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PELIOSIS HEPATIS*

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The disorder which was given the name "peliosis hepatis" by Schoenlank has, in the course of time, been interpreted in various, often diametrically opposed ways. This, together with its rarity, makes reports on this topic desirable.

The gross diagnosis is simple because of the unique appearance of the liver, which contains tiny dark-red spaces filled with liquid or clotted blood. These may be seen through the intact capsule and better on the cut surface. In most instances, there is associated a fatal tuberculosis, usually pulmonary (27 of 31 cases†). Grätzer gave the incidence of peliosis as over 0.2 per cent in his large series of tuberculous cases.

Report of Case

The patient was a man, 57 years of age, who was admitted for treatment of a "chronic putrid lung abscess." He had been in good health until 2 years before admission when he failed to recuperate from a "virus pneumonia" and developed lassitude and progressive anemia requiring transfusions. He lost 70 pounds. (32 kg.). Six months later he coughed up a large amount of brown fetid sputum. From that time he continued to cough and to expectorate foul material. There was slight fever. Further study disclosed a severe hypochromic anemia (hemoglobin, 30 per cent; red blood cells, 2.3 millions), and a consistently positive guaiac test on the stools. Bronchoscopic examination showed pus coming from the apical branch of the left lobe bronchus. X-ray studies revealed a left paravertebral mass which elevated the diaphragm on that side and pressed on the lesser curvature and posterior wall of the stomach. The left kidney was displaced downward; its pelvis was inadequately outlined on pyelographic study. A specimen obtained by an exploratory operation was reported as papillary adenocarcinoma of the kidney. The patient died 8 days later.

Gross Findings

At necropsy (no. 13278) the body was markedly cachectic, with sacral decubitus and gaping left lumbar incision. The right pleural space was completely obliterated by fibrous tissue, save for the apex. Similar

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† In 5 additional cases (Saltykow, Swetschnikow) the cause of death was not listed.

adhesions were seen in the posterior, inferior, and mesial parts of the left pleural sac.

There was a firm, white, solid tumor mass in front of and along the spine, extending from the lower thoracic vertebrae to the sacrum. This mass ensheathed the aorta, the inferior vena cava, and tumorous lymph nodes. Near the surgical incision the mass was necrotic.

Lungs. The lungs were voluminous, fleshy, and water-logged. The bronchi were injected. The left lower lobe bronchus was angry-red and communicated through a left paravertebral sinus with the necrotic retroperitoneal mass. The lung tissue surrounding the sinus tract was fibrotic.

Kidneys. The upper pole of the left kidney was transformed into a necrotic tumor which was continuous with the prevertebral mass. The left adrenal was completely destroyed by this process. The lymph nodes at the hilus of the left kidney and the pancreatic nodes were incorporated in this tumor. The renal vascular pedicle passed through the tumor. The renal vein and its larger intrarenal branches were filled with a pale pink, adherent clot. The portion of the kidney not involved by neoplasm was very pale and had lost its markings on the cut surface. In addition to the sinus leading into the bronchial tree there was a second one, which ran anteriorly to the posterior wall of the stomach, which was adherent to the retroperitoneal tumor.

Stomach. On opening the stomach, neoplastic infiltration was seen on the posterior wall over an area 4 cm. in diameter. The center of this lesion was ulcerated and led into the fistulous tract just mentioned. As evidence of the protracted blood loss through the gastro-intestinal tract, there were blackish patches on the mucosa of the antrum, while the entire small intestine disclosed fine black stippling due to iron pigment in individual villi.

Liver. The liver weighed 1800 gm. and had a rounded edge. Its consistence was normal; its color, pale tan. There were numerous fine, filmy, fibrous adhesions between liver and diaphragm. Many dark purplish spots shone through the capsule. They were seen also on the cut surface where they were determined to be minute cysts filled with clotted or liquid blood. These lesions were scattered irregularly throughout the organ. The lobular centers were readily discernible as injected areas.

The *gallbladder* contained pigment gravel. The portal nodes were involved by tumor.

Additional findings are listed in the anatomic diagnosis: Death 40 days and 8 days after exploratory laparotomies for a necrotic and in-

fected papillary adenocarcinoma of left kidney with destruction of the left adrenal, invasion of prevertebral tissue, left lung, and stomach (bronchial and gastric fistulae); metastasis to left renal, pancreatic, portal, lumbar, and iliac nodes; organizing thrombus of left renal vein; fibrous adhesions around both lungs with partial obliteration of right pleural space; healed perisplenitis and perihepatitis; multiple small hemorrhages of liver (peliosis hepatis); pigment gravel in gallbladder; septic spleen (*Escherichia coli,* enterococcus); mucosal hemosiderosis of gastric antrum and small intestine; osteoporosis; anemia; decubitus; cachexia.

Microscopic Findings

Serial sections of the liver stained with hematoxylin and eosin, van Gieson's, Wilder's reticulum, and Perls' iron stain showed the following:

The blood cysts seen grossly corresponded to foci which were generally sharply outlined and oval or irregular. Spherical foci also were observed. Many, particularly the larger foci, occupied the centers of the lobules and were equidistant from the portal fields. Others failed to show this preference. Occasionally the foci were in close proximity to areas in which the sinusoids were conspicuously dilated. The lesions were filled chiefly with blood and fibrin in various proportions. Frequently these constituents were sharply separated from each other as though the red cells had settled before the onset of coagulation. The white blood cells present tended to accumulate at the margins. They consisted chiefly of lymphocytes with a lesser number of polynuclear and plasma cells. Nowhere was there evidence of organization.

The border of the lesion was formed by liver cells, the spaces having neither a connective tissue wall nor an endothelial lining. In some instances small segments of the wall appeared to be lined by endothelium. However, the latter most likely was merely a remnant of the original sinusoid. Only occasionally did some of the cells forming the rim show regressive nuclear changes and dissociation from their neighbors. The parenchyma surrounding some foci was compressed and appeared to be arranged concentrically. The variations in the composition of the wall or the contents of the lesions were too slight to permit a chronologic differentiation. Lesions in which necrosis and white blood cell accumulation were prominent were encountered very infrequently.

The blood-filled spaces communicated freely with sinusoids. Except for the smallest, they also communicated with central or sublobular veins, where they were located alongside, or as terminal bulbous structures (Figs. 2 and 3). Portal veins and hepatic arteries did not communicate directly with the foci. Frequently two or more peliotic hemorrhages

touched, being separated only by an attenuated septum of compressed liver cells. This might break down, allow for broad communication, and presumably result in cloverleaf and figure-of-eight hemorrhages.

One of the less conspicuous but highly interesting features was the occurrence of atrophic acinar centers containing basophilic mononuclear cells, many of which were typical plasma cells. Russell bodies and iron pigment also were seen occasionally in these areas. The reticulum was condensed and frequently no central vein could be made out. Numerous red cells often surrounded the central core of plasma cells. The atrophic areas occasionally were continuous with a typical peliotic focus.

Uncommon lesions which might be related to the foregoing were small collections of basophilic mononuclear and plasma cells found at the bifurcation of hepatic and sublobular veins, which I refer to as evidence of angular or bifurcational phlebitis. This lesion occasionally was combined with a platelet thrombus.

Many lobular centers were characterized by conspicuous enlargement of liver cell nuclei which I consider as evidence of intralobular regeneration, as did also Ashworth and Reid. The spaces of Disse were discernible and contained red cells in some areas. Some of the portal venous branches were wider than normal. Fatty changes were insignificant.

Discussion

Wagner's case, published in 1861, although lacking histologic data, is recognized as the first record of this condition. A case report of Cohnheim, which many authors consider as an example of peliosis, is probably an unrelated disorder. The cases of Fabris, Umbreit, Lunghetti, and Ugriumow do not seem to fit the picture of peliosis. Of Schrohe's 2 cases, only one, it is felt, can be included. The present report appears to be the first in the English literature.

The great majority of cases are connected with tuberculosis. Of the 3 acceptable instances of peliosis which are not connected with tuberculosis, one had severe cystopyelitis, pyonephrosis, and sprue (Jeckeln); the others had infected carcinoma of the stomach (Hedrén), and of the rectum (Senf). The present case is one of infected renal carcinoma.

In the main, the following five theories with minor variations have been put forward to account for the formation of the blood-filled spaces in the liver:

1. The theory of congenital malformations which become manifest in congested livers (Swetschnikow, Jaffé, Geisler, Meusburger).

2. The theory of vascular varicosities with or without preceding angiitis (Schrohe, Mittasch, Grätzer).

3. The theory of ruptured vessels with or without preceding inflammation (Peltason, Meyer).

4. The theory of primary focal hepatic necrosis followed by hemorrhage (Hedrén, Schoenlank, Senf).

5. The dual origin theory: sharply defined lesions arise from inflamed veins which bulge into necrotic parenchyma. Lesions connecting with capillaries only arise by parenchymal necrosis (Weber).

The discrepancies among the various authors become even more marked when the discussion turns to the presence or absence of an endothelial lining or connective tissue wall, and particularly when communications between peliotic foci and various blood vessels are described. (For details see Table I.) However, it is possible to reconcile the various findings, as was done by Senf. He considered them as stages of one process, initiated by a focal necrosis of liver parenchyma which he illustrated by the following diagram:

Miliary necroses

↙ ↘

Central or peripheral hemorrhages into necrotic area (*e.g.*, accompanying sudden increase of blood pressure in hepatic veins)	Healing (sclerosis)
	↑
Early phase of peliosis →	Thrombosis with organization (resorption)
↓	
Resorption of necrosis, beginning formation of wall around hemorrhage *Intermediary phase of peliosis*	
↓	
Formation of complete fibrous wall and endothelial lining around cavity filled with fibrin *End phase of peliosis*	

The transformation of a focus of necrosis into an area of hemorrhage is something that can be only surmised. A number of authors have resorted to the assumption that a sudden increase in venous pressure is a necessary prerequisite (Meyer, Mittasch, Peltason, Senf). Bouts of coughing, common in pulmonary tuberculosis, combined with massive fibrous pleural adhesions, particularly between lung and diaphragm, are

repeatedly mentioned in this connection. These two factors, which are also present in my case, are frequently invoked to explain the not uncommon accumulation of peliotic foci in the subdiaphragmatic portions of the liver. The "bifurcational phlebitis" in my opinion is a coördinal rather than a causal finding.

With a few exceptions no areas of fresh necrosis were observed in the present case. This, however, does not rule out the possibility that foci of necrosis had been present. These, then, either underwent transformation to peliotic foci, or healed to such an extent that there remained only areas of intralobular liver cell regeneration (Ashworth and Reid) and/or areas of atrophy with inflammatory cells (plasma cells).

I favor the interpretation that the atrophic areas represent healed necrotic foci, but a number of authors have thought that these lesions represented the end-stage of healed hemorrhage. Senf is of the opinion that it might be impossible to say in a given case whether such a scar was preceded by necrosis alone or combined with hemorrhage. Reviews of the literature by Senf and by Weber cite evidence of the frequent occurrence of focal hepatic necrosis in tuberculosis and in other processes in which there is extensive tissue destruction.

From the present case it is possible to assemble pictures showing all stages of development between an atrophic lobular center with plasma cells and a typical peliotic focus. In such a series the lesion would begin as a small hemorrhage around a central vein and end in a characteristic lesion of peliosis containing a number of plasma cells near its periphery. This, however, is ill-founded in view of the clear-cut cases in which focal hepatic necrosis was present.

Summary

Peliosis hepatis is a condition characterized by multiple small cystic blood-filled spaces in the liver. The disorder is quite rare—some 30 cases are known—and occurs predominantly in people dying of tuberculosis.

The more recent publications on this subject emphasize the occurrence of disseminated foci of hepatic necrosis as a prerequisite for the formation of the blood-filled spaces of peliosis of the liver. This seems to be the most appealing explanation.

The present case is the first of this condition to be described in the English literature.

After submission of this paper there appeared E. D. Rosenfeld's report on "Peritoneal Pseudomyxoma," *Arch. Path.*, 1949, **48**, 255–273. Figure 3 shows a typical picture of peliosis of the liver. I am indebted to Dr. Rosenfeld for a tissue block and for information that there was no history of cough, nor were fibrous pleural adhesions found at autopsy in his case.

TABLE I

Acceptable Cases of Peliosis Hepatis

Author	Acceptable cases	Sex	Age	Main disease	Type of communication of blood spaces with other vessels	Wall and lining of blood spaces	Focal degeneration	Organization and healing	Etiologic concept	Remarks
Wagner, 1861	1	F	32	Tuberculosis	With hepatic veins				Congestion is not a causative factor	Case of historical value only
Schrohe, 1899	1	F	31	Tuberculosis	With portal veins	Endothelium and fibrous wall present	Present with engorged sinusoids		Angiectasia of portal veins	Condition related to angiomatosis of cattle; right pleural adhesions
Meyer, 1908	3	M M F	49 25 63	Tuberculosis Tuberculosis Tuberculosis	Small foci with capillaries, large foci with portal veins (case 1); small foci with central veins, large foci with sublobular veins (cases 2 and 3)	No endothelium, no fibrous wall	Present	Early organization (case 3); others are terminal or agonal	Rupture of veins at origin of capillaries	Right pleural adhesions; foci more numerous near convexity or only there (case 3); hemorrhage often independent of necrosis
Hedrén, 1909	1	F	80	Gastric carcinoma		Endothelium in some foci	Present		"Disseminated telangiectasia"; necrosis precedes hemorrhage	Related to disease in cattle
Swetschnikow, 1910	3				Not clearly stated (portal?)				Congenital	
Schoenlank, 1916	1	F	33	Tuberculosis	Some foci break into central veins	No wall, no lining	Present	None; process agonal	Necrosis precedes hemorrhage	Right pleural adhesions; foci more numerous near diaphragm; hyaline thrombi play important rôle; coined name peliosis hepatis because of supposed similarity to peliosis cerebri

TABLE I (cont'd)

Acceptable Cases of Peliosis Hepatis

Author	Acceptable cases	Sex	Age	Main disease	Type of communication of blood spaces with other vessels	Wall and lining of blood spaces	Focal degeneration	Organization and healing	Etiologic concept	Remarks
Mittasch, 1920	1	M	29	Tuberculosis	With sinusoids and sublobular veins	Most foci have endothelium and fibrous wall	Present	Present	Angiectasia subsequent to liver damage	Foci somewhat denser on diaphragmatic surface of liver; thrombi in capillaries secondary to liver damage
Peltason, 1921	2	M F	24 40	Tuberculosis Tuberculosis	Lateral with sublobular veins; terminal with central veins; occasionally with sinusoids only	No definite endothelium; incomplete fibrous wall in larger foci; mainly due to comprest reticulum	None	Present	Rupture of veins	Foci more numerous in upper anterior part of liver; or only there (case 2); no adhesions of right lung (case 1); bifurcational phlebitis leads to obliteration of central veins and cellular scars
Jaffé, 1923	1	M	56	Tuberculosis		Always with endothelial lining	None	None	Diffuse capillary angiectasia, congenital; congestion is additional factor	Related to disease in cattle
Mittasch, 1924	4	M M F M	59 20 30 51	Tuberculosis Tuberculosis Tuberculosis Tuberculosis	With sublobular veins, central veins and sinusoids	Endothelium and fibrous wall in many instances	Present in cases 1 and 2	Foci may heal	Angiectasia; liver necrosis not essential	All 4 cases had extensive right pleural adhesions; the liver foci are more numerous near diaphragmatic surface

Grätzer, 1928	6	M M M F F	44 36 29 23 39	Tuberculosis Tuberculosis Tuberculosis Tuberculosis Tuberculosis Tuberculosis	Central or sublobular veins	Some foci have complete or partial fibrous wall	Never	Present	Angiectasia of inflamed or toxically damaged veins	Foci of vascularized connective tissue with iron pigment in centers may be healed peliosis; bifurcational phlebitis may lead to obliteration of central veins and cellular scars
Geisler, 1931	2	M M	34 56	Tuberculosis Tuberculosis	Foci are dilated central or sublobular veins	Endothelium and fibrous wall	None	None	Angiectasia (perhaps toxic) of congenitally weak vessels	Case 2 has blood spaces in spleen, similar to Cohnheim's case
Meusburger, 1934	1	M	33	Tuberculosis	Sinusoids	Always with endothelium	None		Accepts interpretation of Jaffé	
Jeckeln, 1939	1	F	65	Sprue					Telangiectasia	Severe cystopyelitis; pyonephrosis
Senf, 1939	3	M M F	32 53 37	Rectal carcinoma Tuberculosis Tuberculosis	Central veins, portal veins, or sinusoids only	No lining; no fibrous wall	Present	Present	Necrosis precedes hemorrhage	Bifurcational phlebitis
Weber, 1947	2	M	30	Tuberculosis Tuberculosis	Central veins, sublobular veins or capillaries only; case 2 only with veins	Always with fibrous wall, often with endothelium	Present in case 1	Present in case 1	Dual origin (see fifth theory under Discussion)	Bifurcational phlebitis
Zak, 1948	1	M	57	Renal carcinoma	Central veins, sublobular veins, or sinusoids only	No lining, no fibrous wall	Hardly any	Questionable	Senf's interpretation seems best	Extensive right pleural adhesions; bifurcational phlebitis

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[*Illustrations follow*]

DESCRIPTION OF PLATES

All photomicrographs are from sections stained with hematoxylin and eosin unless otherwise stated.

Plate 1

Fig. 1. Enlarged view of cut surface of liver showing hyperemia of lobular centers and foci of peliosis.

Fig. 2. Terminal position of focus of peliosis on central vein.

Fig. 3. Lateral communication of central vein with peliotic focus. Wilder's reticulum impregnation.

Fig. 4. Inflammatory cells form a cuff around a sublobular vein as it enters the hepatic vein.

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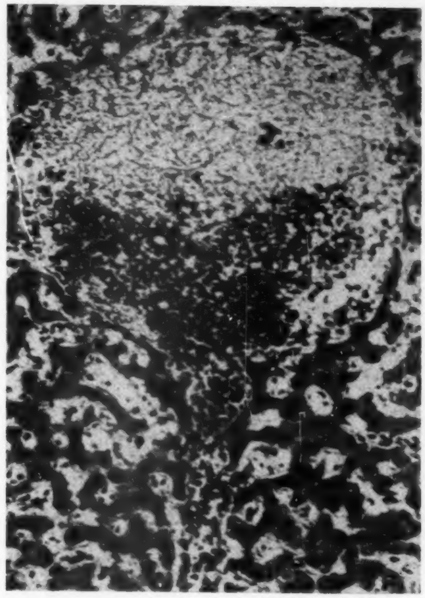

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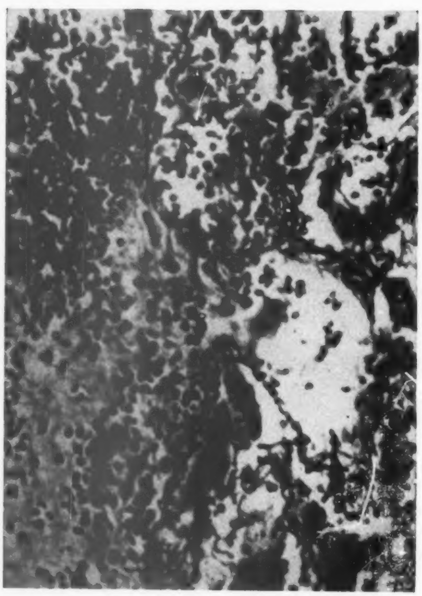

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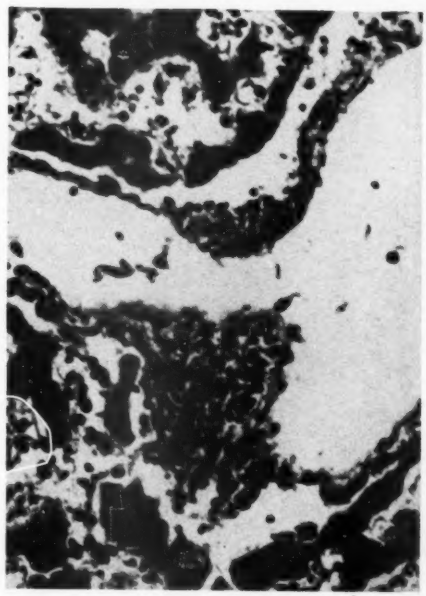

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Plate 2

Fig. 5. A centrolobular scar with inflammatory cells and extravasation of red cells.

Fig. 6. Same area as in Figure 5, showing dense reticulum. Wilder's reticulum impregnation.

Fig. 7. A vascular centrolobular scar with inflammatory cells. The lesion is of considerable size. The central vein has disappeared.

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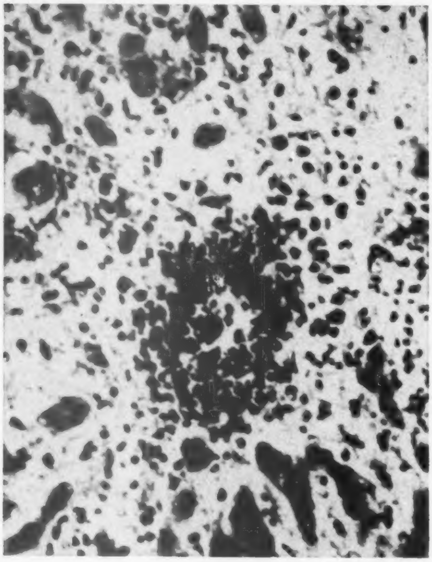
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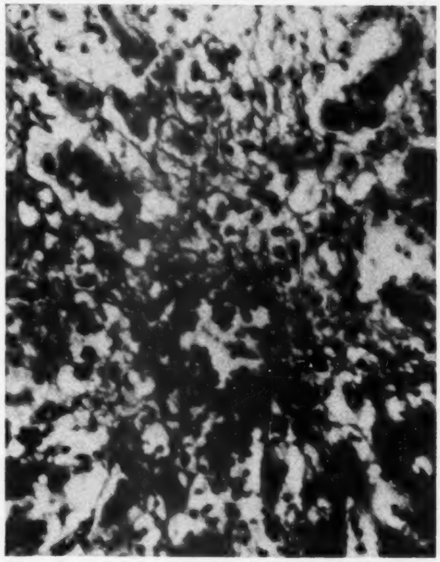

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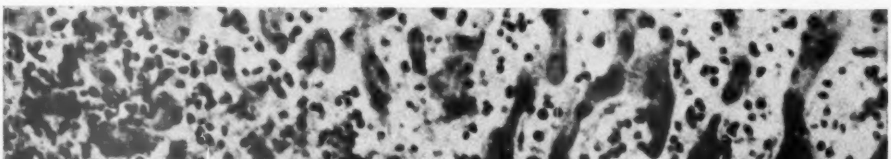

Zak

Peliosis hepatis

NEW FEATURES OF INCLUSION DISEASE OF INFANCY*

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There is much confusion in medical literature concerning the nature and significance of the intranuclear and intracytoplasmic inclusion bodies found in the tissues of infants. This is especially true since inclusion bodies have been observed in several viscera as incidental findings at autopsy. The inclusions have been seen most frequently in the salivary glands of infants, and their resemblance to inclusion bodies observed in the salivary glands of guinea-pigs has been noted. Several excellent reviews of these findings are available,[1-3] and no attempt will be made to record all of them in this paper.

Inclusion bodies in the organs of infants were first described as "protozoan-like" cells by Jesionek and Kiolemenoglou[4] in 1904. Goodpasture and Talbot,[5] in 1921, preferred to recognize these "protozoan-like" cells as a metamorphosis of tissue cells resulting from the effects of an unknown etiologic agent. In addition, they pointed out the similarity of these cells to the cells containing intranuclear bodies of varicella, described in 1906 by Tyzzer.[6] Jackson,[7] in 1920, described "protozoa" in the organs of an infant and discussed their similarity to cells which had been observed in the salivary glands of guinea-pigs. Cole and Kuttner,[8] in 1926, isolated a filterable virus from the submaxillary glands of guinea-pigs in which inclusion-bearing cells could be demonstrated. They were able to infect guinea-pigs with this virus and to demonstrate atypical cells with nuclear inclusions in the infected animals. Farber and Wolbach[1] in 1932, Vidari[9] in 1940, Kinney[2] in 1942, Cappell and McFarlane[3] and Kalfayan[10] in 1947 described and discussed cases of this disease, which has gradually come to be known as "inclusion disease of infancy." It would appear, however, that this disease is not limited to infants since Von Glahn and Pappenheimer[11] reported an adult case in 1925. Recently McMillan[12] stated that the identical histologic picture has been described in the tissues of adults in 8 reported cases; and he reported a case of his own, bringing the total to 9 cases.

Report of Case

History. D. A. H., a 6-weeks-old white male, entered Duke Hospital for the first time on July 3, 1947, with the chief complaint of diarrhea and an enlarged spleen. The patient had been a full-term, spontaneously delivered infant, jaundiced at birth. The jaundice disappeared within 5 days. On his tenth day, the patient had been

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admitted to another hospital because of severe hemorrhage from the stump of the umbilical cord and was transfused with blood donated by the father. During the third week of life the patient began to have 10 to 12 diarrheal stools per day which were described as foul and greenish brown. The mother stated that blood was present in the stools on two occasions. There was neither nausea nor vomiting. A local physician observed that the spleen was enlarged. Medication failed to improve the child's condition, and the weight dropped from 6 to 5 lbs.

The mother was 20 years of age, and there was no history of previous transfusions. She had had one uncomplicated pregnancy 3 years previously, and that child was living and well. Her blood, as well as that of the patient, was type O, Rh positive. The blood type of the father was unknown.

Physical Examination. On examination, the temperature was 38.2° C., pulse was rapid and regular, and respirations were 30 per minute. The patient was a poorly nourished and moderately dehydrated infant with severe excoriations over the buttocks and scrotum. Behind the right ear there was a small cutaneous abscess. The liver was palpable 1 fingerbreadth below the right costal margin, and the spleen was palpable at the level of the umbilicus.

Additional Clinical Findings. The red blood cell count was 3.5 million, with a hemoglobin content of 9.5 gm. The white blood cell count was 13,500 with 7 per cent juvenile forms, 34 per cent staff forms, 28 per cent segmented forms, 10 per cent small lymphocytes, 18 per cent large lymphocytes, 3 per cent monocytes. The urine was negative for albumin, sugar, and acetone. The stools were formed, greenish brown, and negative for mucus, pus, parasites, and ova. The guaiac and benzidine tests were negative. The CO_2-combining power was 21.7 volumes per cent. A blood culture revealed no growth, and a stool culture was negative for intestinal pathogens. The Kahn, Kline, Wassermann, and Mazzini tests were all negative.

Course in Hospital. The patient received 60 cc. of 1/6 molar lactate solution on the day of admission, and by the second hospital day the CO_2-combining power had risen to 24 volumes per cent. He again received 125 cc. of 1/6 molar lactate by clysis and was started on tea and Ringer's solution by mouth. The stools continued to be formed and were described as yellow. The patient expired on the second hospital day.

Clinical Impression. 1. Dehydration, secondary to diarrhea of newborn. 2. Splenomegaly, cause undetermined, probably on an infectious basis.

Post-mortem Examination

Post-mortem examination was performed 13 hours following death. The body weighed 2580 gm. and measured 31 cm. from crown to rump and 49 cm. from crown to heel. There was generalized icterus, and pinpoint petechiae were scattered over the hands and feet and anteriorly over the trunk. The buttocks and scrotum were excoriated and crusted. A raised purplish red ulcerated lesion, 1 cm. in diameter, was observed behind the right ear.

There were scattered small hemorrhages in the muscles of the flanks bilaterally. No fluid was present. The spleen and liver were enlarged. Three to 4 cc. of clear fluid were found in each pleural cavity. No adhesions were present.

The ductus arteriosus was patent to a small probe as was the foramen

ovale. The epicardium, endocardium, myocardium, and valves showed no gross lesions.

No lesions were observed in the lungs.

The spleen weighed 42 gm. and was enlarged to about three times the normal size. The capsule had a granular or wrinkled appearance. The organ was firm and the cut surface was dark purplish red, with indistinct malpighian bodies.

There were eight small hemorrhages observed in the mucosa of the small intestine, and Peyer's patches appeared enlarged. The entire large intestine showed considerable mucosal congestion with scattered small hemorrhages. The mesenteric lymph nodes were enlarged and hard and rubbery. Areas of hemorrhage similar to those observed in the large bowel were present in the gastric mucosa. The duodenum was not remarkable. The gallbladder contained viscid yellowish white bile. The mucosa of the gallbladder revealed scattered pin-point petechiae. The pancreas showed no lesions.

The liver weighed 180 gm. The capsule was smooth, and on cut section the architecture appeared normal; but some areas appeared to contain an increased amount of bile pigment.

Each adrenal weighed 5.5 gm., and no lesions were observed grossly.

Each kidney weighed 21 gm., and no lesions were found. Several petechial hemorrhages were found in the bladder in the region about the trigone. The prostate showed no gross lesions. Both testicles showed scattered hemorrhages.

Several small petechial hemorrhages were seen in the esophageal mucosa. The salivary glands were not observed or removed, as the nature of the disease was not realized at the time of autopsy.

The aorta showed no lesions. The retroperitoneal and inguinal lymph nodes were enlarged and firm, and their cut surface was light brown.

The bone marrow appeared paler than is normal.

In the brain there was an area of hemorrhage involving the region of the caudate nucleus and surface of the thalamus on the right.

Staphylococcus albus (hemolytic and nonhemolytic) was cultured from the left lung. The nature of the disease was not suspected at the time of autopsy, and consequently virus studies were not attempted.

Microscopic Examination

The most conspicuous findings were large (15 to 22 μ) cells with prominent nuclei and a bluish granular cytoplasm. A more detailed study revealed that these features were due to intranuclear and intra-

cytoplasmic inclusions. In studying the inclusions, many methods of staining were employed, including those designated as Giemsa, Wright, MacCallum (bacterial), Dieterle, Delafield hematoxylin, Levaditi, Ziehl-Neelsen, Wilder, Mallory's aniline blue, Mallory's phloxine and methylene blue, iron, Heidenhain, Gram-Weigert, methylene blue, phosphotungstic acid hematoxylin, and iron hemofuscin. It was found that the hematoxylin and eosin stain was best suited for the demonstration of the inclusion bodies. The hypertrophied cells could easily be identified under low magnification. All tissue was fixed in Zenker-formalin and in formalin solutions.

With the hematoxylin and eosin stain the *intranuclear* inclusions appeared as a single, round, oval, or kidney-shaped, acidophilic-staining, homogenous material having a rather definite outline and measuring from 2 to 10 μ in diameter (Fig. 9). The chromatin in most of the nuclei was marginated, thus giving the nuclei the characteristic "owl's eye" or "bird's eye" appearance. The affected nuclei varied from 7 to 13 μ in diameter.

The *intracytoplasmic* inclusions showed considerable variation. In some cells they appeared as fine powdery, acidophilic material; in others, they were greatly increased in mass (8 μ) and, in general, basophilic. As the inclusions increased in size, they became more basophilic, although some large intracytoplasmic inclusions in the brain maintained acidophilic properties. The intracytoplasmic inclusions were always multiple in a given cell and tended to be uniform in size in that cell. They did, however, occasionally show marked variation in a single cell. These inclusions were often more concentrated in the pole of the cell opposite the nucleus, but at times they were diffusely scattered throughout. In the cytoplasm of some inclusion-bearing cells, single or multiple vacuoles appeared.

Many cells contained intranuclear inclusions without intracytoplasmic inclusions, but every cell with intracytoplasmic inclusions contained an intranuclear inclusion with the exception of a few cells in which no nucleus could be seen and in which numerous intracytoplasmic inclusions were observed. These latter cells resembled the so-called pseudocysts of Toxoplasma.

In order to simplify description, the characteristic hypertrophied cells containing inclusion bodies will hereafter be referred to as "inclusion cells." The use of such a term is justified, since, in this disease, no one has as yet determined the nature of these inclusions, nor their relationship to the host cells.

Heart. Large inclusion cells were found singly, or in twos or threes, intimately associated with cardiac fibers (Fig. 1) or lying between them. Most of the cells were elongated, but some were rounded. In addition, inclusion cells were found in capillaries and in capillary walls.

Lungs. The pulmonary spaces contained blood, pigment-laden macrophages, and a few polymorphonuclear leukocytes, as well as a few inclusion cells. Inclusion cells were observed also in bronchi, alveolar spaces, and within capillaries in alveolar walls. Gram-positive cocci were found in clusters.

Liver. The architecture of the hepatic lobules was unaltered. Foci of extramedullary blood formation were observed in the sinusoids. Inclusion cells could be identified in bile duct epithelium, in sinusoids, in the stroma of the portal areas, and questionably in hepatic cells. The liver cord cells about the central veins contained bile pigment, and the Kupffer cells contained brownish material that stained positively for iron.

Spleen. The spleen showed marked proliferation of reticulo-endothelial cells with compression of the malpighian bodies and apparent diminution in the number of small round cells in the pulp. Inclusion cells were moderately numerous in sinusoids, in sinusoidal walls, and within the lumina of the vessels in the trabeculae.

Kidneys. There were numerous inclusion cells lining the proximal convoluted renal tubules (Fig. 2), and they appeared definitely to be altered epithelial lining cells. A few inclusion cells were free in the lumina of tubules, and some were lining loops of Henle as well as collecting tubules.

Stomach. Small superficial mucosal hemorrhages were present in the stomach. Numerous inclusion cells were found in the mucosal glandular epithelium (Fig. 3) and occasionally in the endothelial lining and within the lumina of submucosal vessels.

Intestines. Both the small and large bowel showed mucosal hemorrhages. Inclusion cells were difficult to find in the epithelium, but were numerous in the mucosal capillaries and in the submucosal vessels.

Mesenteric Lymph Nodes. The mesenteric lymph nodes, as those elsewhere, showed reticulo-endothelial hyperplasia and a moderate number of polymorphonuclear leukocytes in the sinusoids. An occasional inclusion cell was seen in the sinusoids as well as in vessels about the periphery of the nodes.

Skin. The section from the excoriated lesion of the buttock presented a necrotic epidermis with a highly vascular corium and a mod-

erate infiltration consisting mostly of round cells and plasma cells. The dilated capillaries contained inclusion cells in their lumina, in their intimal linings, and in their adventitia. Similar cells were seen in sweat gland ducts (Fig. 4) and appeared to have arisen from the lining epithelial cells. The section from the lesion behind the right ear revealed the lesion to be an infected epidermoid cyst with suppuration and destruction of most of the cyst wall. About the periphery, there was a chronic inflammatory process with granulation tissue. In this peripheral area, numerous inclusion cells were seen in vessels, lining vessels, and in stromal tissues (Fig. 21).

Endocrine Glands. In the anterior lobe of the pituitary body there were numerous inclusion cells that appeared to arise from the chromophobe cells (Fig. 6). A number of inclusion cells were observed lining thyroid acini. Several inclusion cells were seen in the adrenal cortex. In the periadrenal tissue, numerous inclusion cells could be seen in vessels (Fig. 20), lining vessels, about vessels, and in the fat which was fetal in type. Many inclusion cells were present in islets of Langerhans, and a few were seen in acini.

Testes. It was noted that inclusion cells were present in the seminiferous tubules, both at the periphery and in the lumen. Similar cells were seen to be free in the tubules of the epididymis, and some were present within vessels (Fig. 19).

Umbilical Vein. There was marked thickening of the intima of the umbilical vein with hyalinization and calcification. In the adventitia, there was an infiltration of round cells throughout a highly vascular connective tissue. Almost every vessel, regardless of size, contained one or more inclusion cells (Fig. 18) in or about it.

Salivary Gland. The only salivary gland tissue that was available for study was located in the tongue. No inclusion cells could be found.

Bones. A normal epiphyseal line was present in the bones examined. Within the marrow cavity, which was full of normal elements, a few inclusion cells were seen (Fig. 5). These appeared to be related to endothelium-lined sinusoids.

Brain. In the region of the basal ganglia on the right, there was extensive intracerebral hemorrhage (Fig. 7) with marked edema and necrosis of the adjacent tissue. Also present were numerous inclusion cells (Fig. 8) exhibiting transition stages which will be described in a later paragraph. The inclusion cells were numerous in perivascular areas and were in association with a few round cells. The host cell here was thought to be the microglial cell. A few gitter cells and pigment-

containing cells were found. In a few vessels in various areas of the brain, inclusion cells were seen. One such cell was present in the choroid plexus, and one was free in the fourth ventricle. A number of microscopic hemorrhages were observed in the granular layer of the cerebellum, but inclusion cells could not be found in that region.

Comment

This case is presented as another example of "inclusion disease of infancy." The structure of the "inclusion cells" is similar to that of affected cells in previously described cases. There are, however, several unusual features which deserve special comment.

The distribution of the lesions in this case is more diffuse than in any previously reported. There have been 23 cases reported in which inclusion cells were observed in a single organ, and 14 cases in which such cells were seen in more than one organ. Table I presents the sites of the lesions, with the exception of the parotid gland, in the 14 cases in which inclusion cells were seen in more than one viscus and the sites of the lesions in the case reported here. It will be noted that this is the first reported case in which numerous inclusion cells have been described in the brain, heart, bone marrow, sweat glands, pituitary body, and testes. Kinney[2] reported the presence of a single inclusion in the brain. It is also noted that inclusions were present in host cells derived from all three primary germ layers and from several organ systems.

The most impressive lesions were those of the reticulo-endothelial system. Inclusion cells were present in vessels, sinusoids, and stromal tissues throughout essentially all organs. This involvement apparently accounted for the numerous small hemorrhages and for the cerebral hemorrhage which was interpreted as the immediate cause of death. All endocrine glands were found to contain inclusion cells, except the parathyroid glands which were not found. No clinical or morphologic evidence of endocrine dysfunction was noted.

The morphologic character of some of the inclusion cells warrants comment. In the description of the intracytoplasmic inclusions, it was noted that single or multiple vacuoles were observed in some of the cells. This was most striking in the brain lesion where such vacuoles were a prominent feature of the inclusion cells. In many hypertrophied cells containing distinct intranuclear inclusions, a large "bubble-like" area of vacuolization or rarefaction was present in the cytoplasm. This "bubble" had apparently pushed the nucleus, together with its inclu-

TABLE I

Reported Cases with Inclusion Bodies Found in Several Viscera

Observers	Lungs	Liver	Kidneys	Pancreas	Thyroid	Endothelium	Salivary glands	Intestines	Spleen	Adrenals	Epididymis	Testes	Sweat glands	Heart	Brain	Bone marrow	Pituitary body
Jesionek and Kiolemenoglou,[4] 1904	+	+	+														
Pisanò,[13] 1910	+	+	+														
Smith and Weidman,[14] 1910	+	+	+														
Goodpasture and Talbot,[5] 1921	+	+	+														
Von Glahn and Pappenheimer,[11] 1925	+	+				+		+									
Walz,[15] 1926	+	+	+	+	+												
Wagner,[16] 1930	+	+	+	+	+		+				+						
Farber and Wolbach,[1] 1932	++	++	++	++	+												
Vidari,[9] 1940	+	+	+	+	+												
Kinney,[2] 1942	+	+	+			+									+*		
Cappell and McFarlane,[3] 1947	++	++	++	+													
Kalfayan,[10] 1947	+	+	+	+	+		+		+	+							
Worth and Howard, 1948	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+
Distribution of inclusions in the various organs	15	15	14	8	6	3	2	2	2	2	2	1	1	1	2*	1	1

* Kinney[2] found a single intranuclear inclusion in the brain.

sion, toward one pole of the cell (Figs. 10 and 11). In such cells, intracytoplasmic inclusions were not present in the cytoplasm surrounding the area of rarefaction, although fine dust-like acidophilic particles could be found in the "bubble." This cell type has not been previously described, but there have been descriptions of a spongy, foamy structure in the cytoplasm of inclusion cells.[8,10] In other inclusion cells the intracytoplasmic inclusions were larger and more distinct within the area of rarefaction, and the nucleus with its inclusion was flattened against the cell membrane (Figs. 12 and 13). In some cells with a nucleus of this type, the cytoplasm contained numerous large, distinct acidophilic inclusions which were arranged in a circular manner with the convexity toward the cell membrane (Fig. 14). In such cells, no distinct area of rarefaction could be seen. Since these features are not seen in inclusion cells in epithelial structures, it is thought that an area of rarefaction in the cytoplasm is not a necessary antecedent for the development of intracytoplasmic inclusions. These unusual cells are interpreted as indicating that the host cell has phagocytic powers which have become manifest, and that intracytoplasmic inclusions can be formed in the area of ingested material. It is thought that this phagocytic property of the host cell may be responsible for the acidophilic character of some of the intracytoplasmic inclusions, and for the unusual arrangement of the nucleus with its inclusion and of the intracytoplasmic inclusions.

In the brain lesion there were observed inclusion cells that were apparently phagocytizing other inclusion cells (Figs. 15 and 16). So far as can be determined, this phenomenon has never been described. Both the phagocytizing cell and the ingested cell contained intranuclear and intracytoplasmic inclusions.

Another interesting type was the inclusion cell with two or three nuclei, each containing a single, large acidophilic inclusion (Fig. 17). No cell was found with more than three nuclei, although Kalfayan[10] reported as many as eight nuclei in a single hypertrophied cell.

Inclusion bodies are generally accepted as evidence of virus disease, although they are occasionally found in cases in which it is reasonably certain that no viruses are present. In addition, Olitsky and Harford[17,18] and Pappenheimer and Maechling[19] have produced, by means of chemical substances, inclusion bodies resembling those found in virus diseases. Anderson[20] found that by using viruses that form recognizable intracellular inclusions she could demonstrate cytologic evidence that individual cells may be invaded by, and become hosts to, two different viruses at the same time. Such observations serve to

complicate the identification of virus diseases by the demonstration of inclusion bodies. Cappell and McFarlane[3] have pointed out, however, that the salivary gland virus diseases of rodents and monkeys are the only known entities which exhibit cells with cytomegaly and intranuclear and intracytoplasmic changes identical with those observed in organs of infants.

The inclusion cells observed in this case are thought to be similar to those described and depicted in cases of salivary gland virus disease of guinea-pigs.[21,22] The nature of the disease in this case was not recognized at the time of autopsy; therefore, no virus studies were undertaken.

Summary

In the study of the case of inclusion disease which forms the basis of this paper, "inclusion" cells were identified in almost all of the viscera.

Specific lesions in the brain, heart, bone marrow, sweat glands, pituitary body, and testes are reported for the first time.

This is the first reported case of inclusion disease in an infant in which the disease has been implicated as the probable cause of death.

Some inclusion-bearing cells had peculiar areas of rarefaction in their cytoplasm. Other cells were interpreted as showing an apparent phagocytosis of hypertrophied cells with inclusions by other similarly affected cells.

We are indebted to Dr. Margaret G. Smith for helpful opinions concerning the inclusions.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

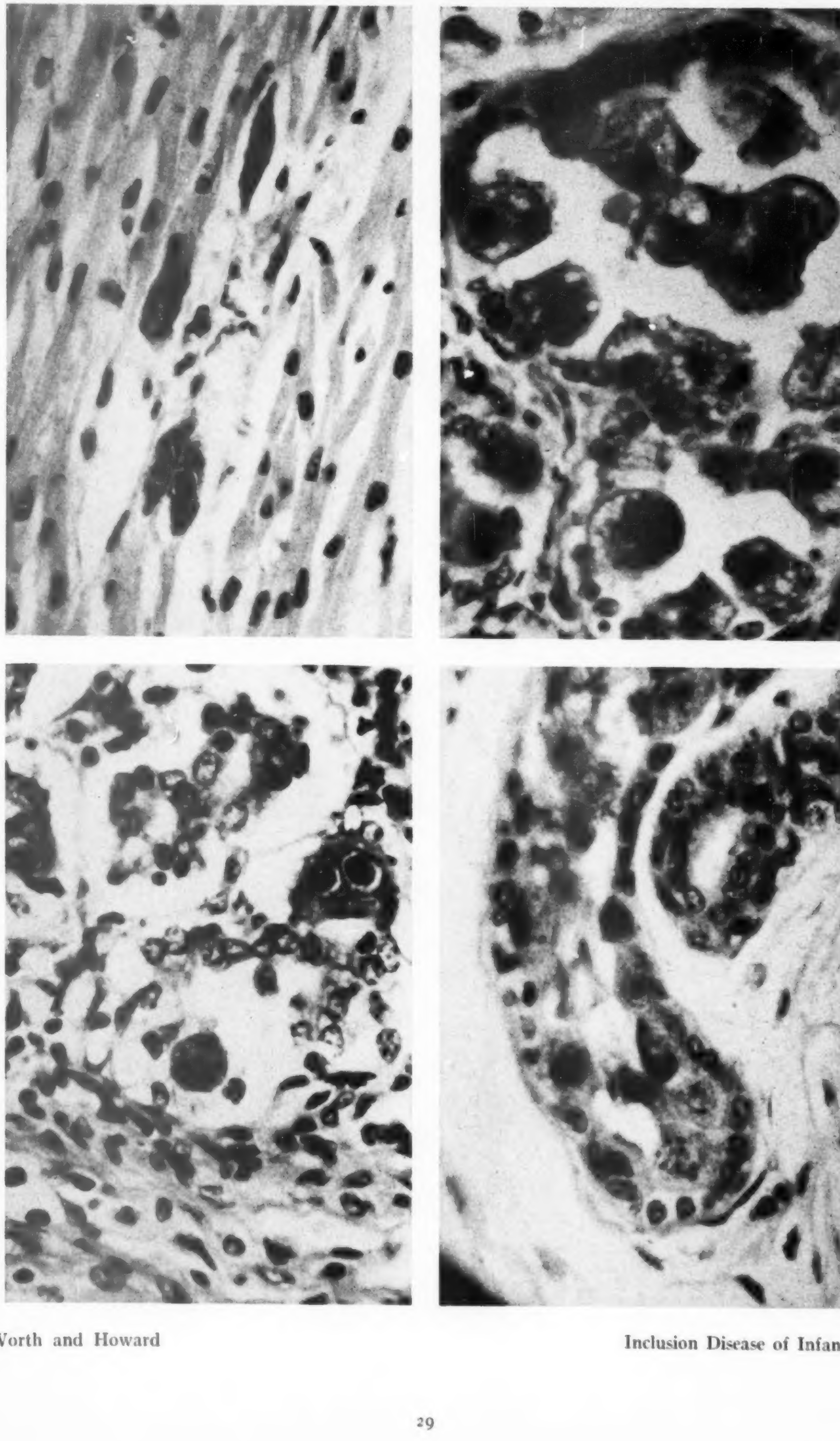
PLATE 3

FIG. 1. Heart. Photomicrograph illustrating the manner in which the inclusion cells appear to take origin from the myocardial fibers. Hematoxylin and eosin stain. × 816.

FIG. 2. Kidney. Intranuclear and intracytoplasmic inclusions. Hematoxylin and eosin stain. × 816.

FIG. 3. Stomach mucosa. Intranuclear and intracytoplasmic inclusions. As in Figure 2, the "owl's eye" appearance caused by the marginated chromatin can be seen. Hematoxylin and eosin stain. × 816.

FIG. 4. Sweat gland. The inclusion cells appear to take origin from the ductal epithelium. Hematoxylin and eosin stain. × 816.



1 2 3 4

Worth and Howard Inclusion Disease of Infancy

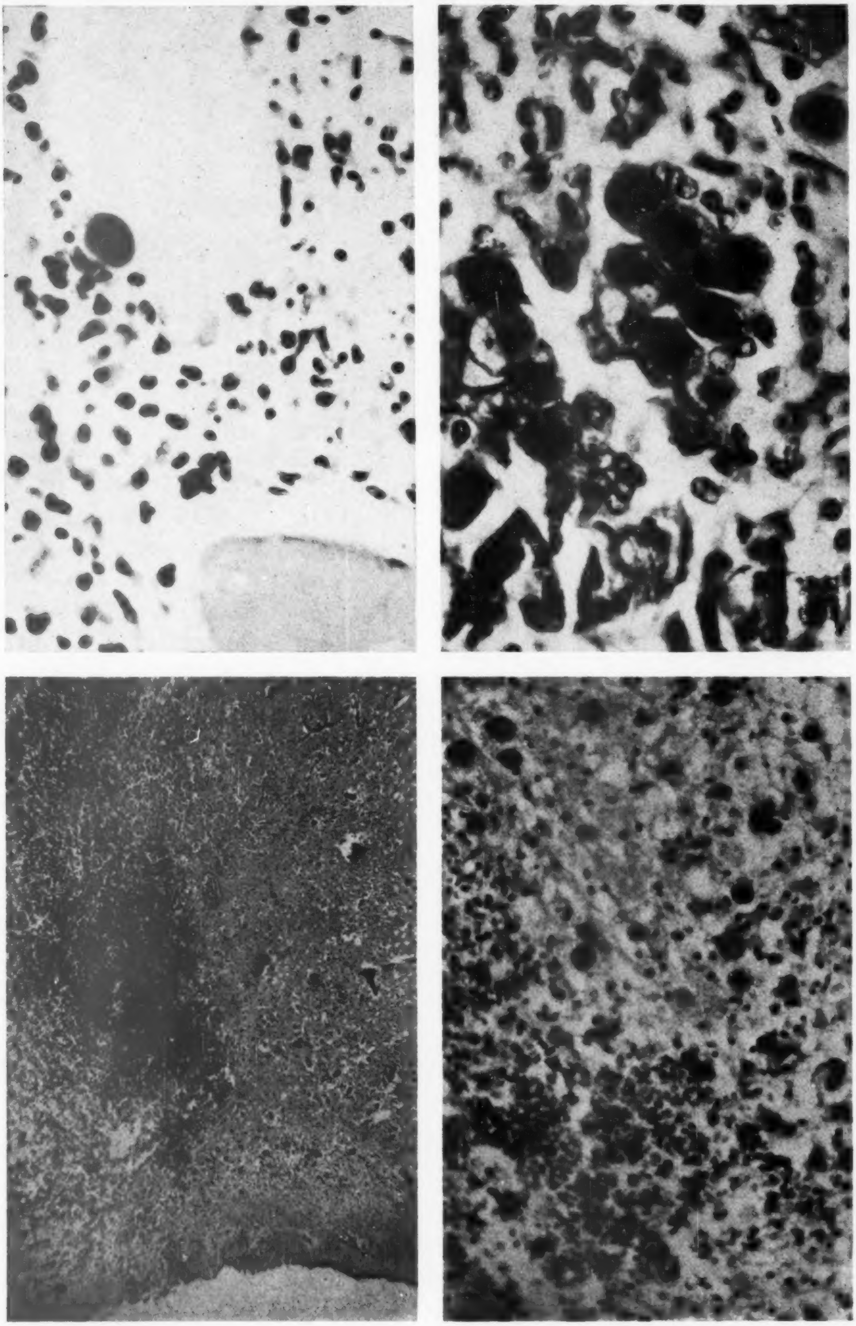
PLATE 4

FIG. 5. Bone marrow. Single inclusion cell. Hematoxylin and eosin stain. × 816.

FIG. 6. Pituitary body. A cluster of inclusion cells. Hematoxylin and eosin stain. × 816.

FIG. 7. Cerebrum. Low-power photomicrograph taken in the region of the right basal ganglia showing extensive hemorrhage and numerous inclusion cells. Hematoxylin and eosin stain. × 70.

FIG. 8. Higher power photomicrograph of Figure 7 illustrating area of hemorrhage and close association with inclusion cells. Hematoxylin and eosin stain. × 162.



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Worth and Howard

Inclusion Disease of Infancy

Plate 5

Fig. 9. Cerebrum. Small intranuclear inclusion, unaccompanied by extreme hypertrophy of the host cell, and absence of intracytoplasmic inclusion. This was one of the earliest stages observed. Hematoxylin and eosin stain. × 1680.

Fig. 10. Cerebrum. A "bubble," or area of rarefaction or vacuolization, has appeared and is literally pushing the nucleus with its large single inclusion to one side. Hematoxylin and eosin stain. × 1680.

Fig. 11. Cerebrum. The "bubble" has expanded to its fullest extent, and the nucleus together with its inclusion is flattened against the cell membrane. Hematoxylin and eosin stain. × 1680.

Fig. 12. Cerebrum. Acidophilic intracytoplasmic granules have begun to appear within a "bubble." A few small granules also appear outside of the area of rarefaction. Hematoxylin and eosin stain. × 1680.

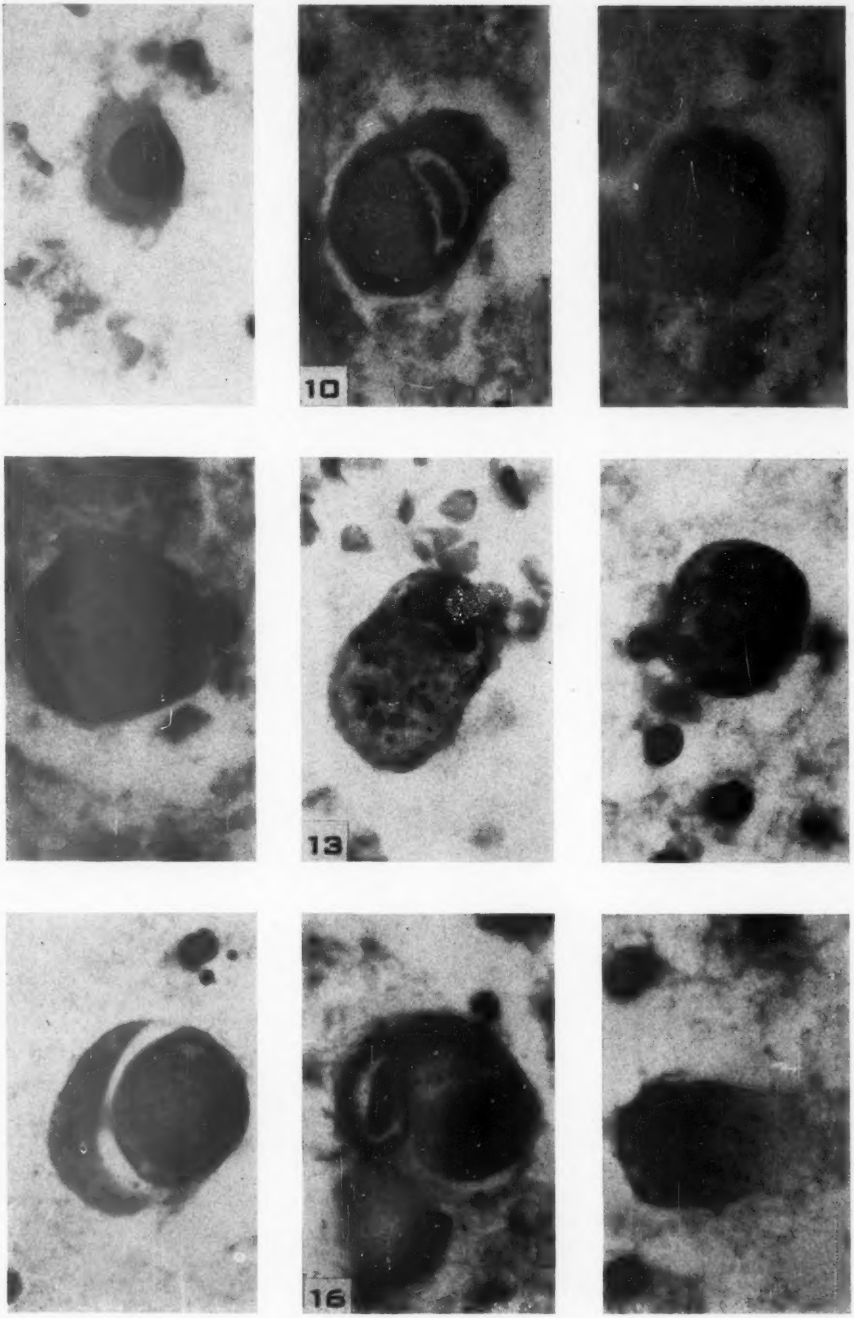
Fig. 13. Cerebrum. Intracytoplasmic inclusion bodies have developed more fully. Area of rarefaction can still be seen. Hematoxylin and eosin stain. × 1680.

Fig. 14. Cerebrum. The acidophilic intracytoplasmic inclusions are arranged in a circular pattern, probably having been limited in distribution by a preexisting "bubble." Hematoxylin and eosin stain. × 1680.

Fig. 15. Cerebrum. An apparent phagocytosis of one inclusion cell by another. Intranuclear and intracytoplasmic inclusions are present in both cells. Hematoxylin and eosin stain. × 1680.

Fig. 16. Cerebrum. One inclusion cell has completely engulfed another similarly affected cell. Hematoxylin and eosin stain. × 1680.

Fig. 17. Cerebrum. A binucleated inclusion cell. Each nucleus has a single acidophilic inclusion. "Orbital bodies" are seen as small condensations of the marginated chromatin. Hematoxylin and eosin stain. × 1680.



Worth and Howard Inclusion Disease of Infancy

PLATE 6

FIG. 18. Umbilical vein. Inclusion cell in the intima of an adventitial vessel. Hematoxylin and eosin stain. × 790.

FIG. 19. Epididymis. Inclusion cell in the lumen of a stromal vessel. Hematoxylin and eosin stain. × 790.

FIG. 20. Periadrenal tissue. Multiple inclusion cells in the lumen of a vessel. Hematoxylin and eosin stain. × 790.

FIG. 21. Subcutaneous nodule. Inclusion cells in an area of chronic inflammation. Hematoxylin and eosin stain. × 790.

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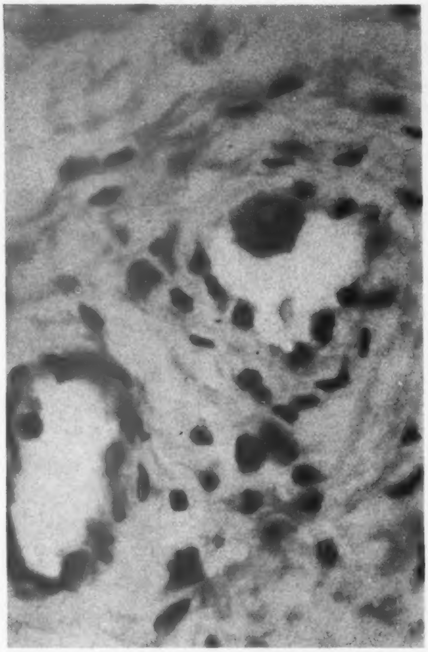

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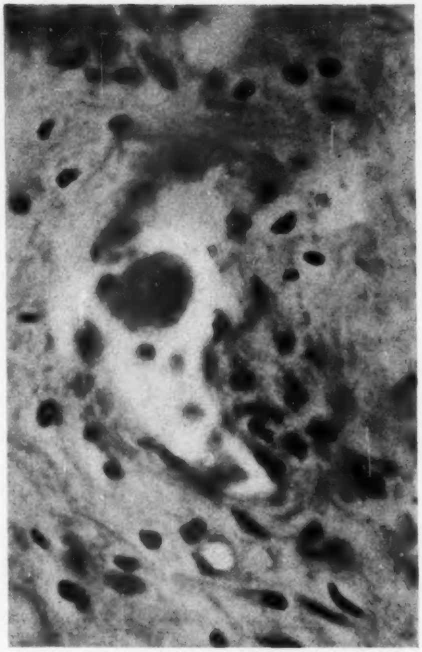

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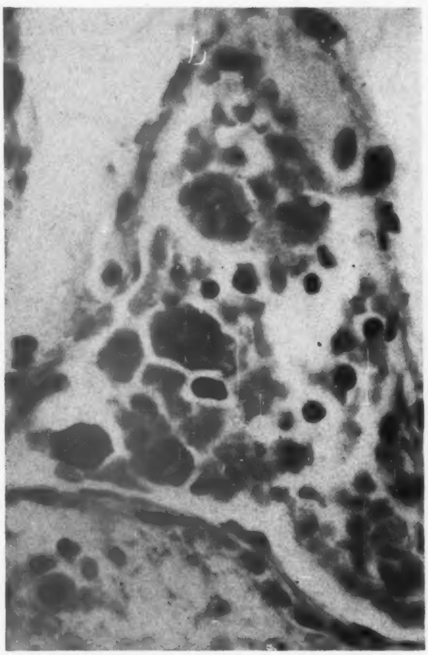

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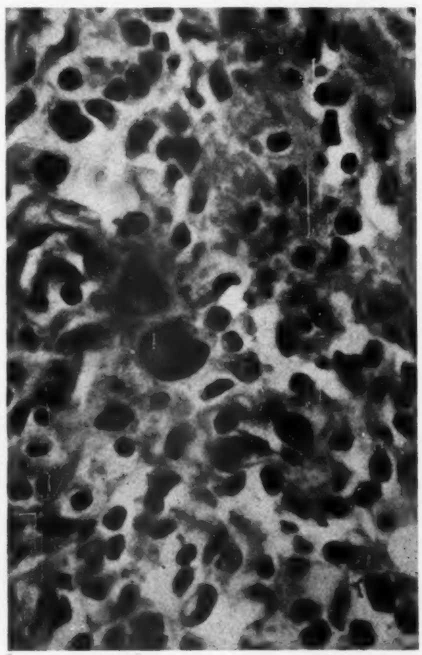

Worth and Howard Inclusion Disease of Infancy

CHRONIC PANCREATITIS AND LITHIASIS
II. Pathology and Pathogenesis of Pancreatic Lithiasis*

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The pathogenesis of calculi occurring in the ducts of the pancreas and of diffuse calcification in the gland has been difficult to explain. For a long time it was thought that calcium was present in such minute quantities in the acinar secretion that it was improbable that calcium salts would be precipitated in normal juice; therefore other mechanisms, such as inflammatory factors, were considered necessary. That chronic pancreatitis is often complicated by the presence of calculi has been emphasized by Comfort, Gambill, and Baggenstoss.[1] In a previous report we presented the clinical and diagnostic features together with a brief outline of the pathologic findings in a group of 22 cases of pancreatic calculi or calcification occurring in a series of 35,000 autopsies.[2] We now have a total of 26 instances of the disease in a series of 36,000 consecutive autopsies performed between September 25, 1925, and March 16, 1948. The histologic details, their relationship to the precipitation of calcium stones, the physical chemistry involved in the formation of calculi, and other pertinent data remain to be discussed. In addition, the results of post-mortem roentgenographic examination of 200 pancreases are included.

Since July, 1946, a careful search has been made for stones in the pancreas. There have been 10 examples in 3,000 autopsies (0.33 per cent); previous to this there were 16 in 33,000 autopsies (0.05 per cent) over a period of 21 years. Because 9 of the 10 recent cases were associated with alcoholism, compared to only 5 of 16 in the preceding period, we believe there has been an actual increase in patients with calculi which parallels the increase in cirrhosis and alcoholism already noted. No doubt a careful examination of the pancreases at necropsy would have increased the total diagnosed before 1946, especially in the years following the repeal of the Volstead Act.

As stated in a previous publication,[2] not one of the first 22 patients was diagnosed correctly by the clinicians. During the past year, of the 4 additions to the list, one was so diagnosed. A study of this last group adds nothing new to the discussion of the clinical aspects and diagnosis.[2] Table I gives the complete data on all 26 patients in regard to

* Received for publication, October 11, 1948.

TABLE I

Clinical and Pathologic Data on 26 Cases of Pancreatic Lithiasis

No.	Age	Sex	Solitary stone	Multiple stones or calcification	Alcoholism	Fatty liver	Cirrhosis	Diabetes	Abdominal pain	Weight loss	Pulmonary tuberculosis	Icterus
1	36	F		Yes	Yes	+++	+	No	Yes	Yes	No	Yes
2	86	F	Yes		No	–	–	Yes	No	Yes	No	No
3	55	F	Yes		No	–	–	No	Yes	Yes	No	No
4	22	F		Yes	No	–	–	Yes	No	Yes	Fatal	Yes
5	39	F		Yes	Yes	++++	++	No	Yes	Yes	No	Yes
6	58	M		Yes	Yes	+++	++	No	No	Yes	Yes	Yes
7	78	M	Yes		No history	++	–	No tests done	No history	?	No	No
8	55	M	Yes		No history	+	+++	No tests done	No history	Yes	Fatal	Yes
9	52	M		Yes	Yes	++	–	Yes	No	Yes	No	No
10	55	F		Yes	No	–	–	Yes	Yes	Yes	No	Yes
11	34	F		Yes	Yes	++	–	No	No	No	No	No
12	44	F		Yes	No	++	–	Yes	No	Yes	No	No
13	37	F		Yes	No	–	–	Yes	No	Yes	No	Yes
14	62	M		Yes	Yes	++	+	No	Yes	No	No	Yes
15	77	M		Yes	No	–	–	No	No	No	No	No

16	80	M		Yes	Yes	++	Biliary cirrhosis	No	Yes	Yes	No	Yes
17	46	M	Yes		?	–	–	No	Yes	No	No	Yes
18	79	M	Yes		Yes	++	+++	No	No	Yes	No	Yes
19	76	M		Yes	No	–	Biliary cirrhosis	No	No	Yes	No	Yes
20	42	M		Yes	Yes	++	+++	Yes	No	Yes	Fatal	No
21	35	F		Yes	Yes	+++	+++	No	No	Yes	No	Yes
22	52	F		Yes	Yes	++++	+	No	No	Yes	Fatal	No
23	43	F		Yes	Yes	++++	+	No	Yes	No	No	Yes
24	46	F		Yes	Yes	+	+++	Yes	No	No	No	Yes
25	60	M		Yes	No	–	–	No	Yes	Yes	No	No
26	37	F		Yes	Yes	+++	++	No	Yes	Yes	No	Yes
Totals			6	20	14	17	14	8	10	19	5	16

alcoholism, diabetes, and other clinical features. A summation of certain data of interest in this table is presented in Table II. A history of alcoholism was more prevalent (14 of 20) in those with multiple stones than in those with solitary stones (1 in 6). Diabetes mellitus was seen more often in the non-alcoholic (5 of 11) than in the alcoholic group (3 of 15). This indicates that if a person addicted to alcohol develops concretions in the pancreas, there is more than an even chance that they will be multiple. But, as noted in the group with chronic pancreatitis, the alcoholic patient does not seem to have as high an incidence of diabetes as the non-drinker with pancreatic lithiasis.

TABLE II

The Occurrence of Solitary and of Multiple Calculi in Patients With and Without Diabetes and Alcoholism

	History of alcoholism	Alcoholism and diabetes	No history of alcoholism	Diabetes in non-alcoholics
Multiple stones, 20	14	3	6	4
Solitary stones, 6	1	0	5	1
Totals	15	3	11	5

PATHOLOGY

Gross Observations. Because there are other excellent descriptions, only a few aspects of the gross changes need be discussed. The location of the calculi in the ducts is of interest. Sixteen were located definitely enough to chart on an outline of the duct system (Fig. 1). The remainder were widespread or indefinitely described by the prosector. The favored site for calculi is in the duct of Wirsung within 2 to 4 cm. of the ampulla of Vater. It is possible that they become impacted at this point after being carried down from the body or tail, rather than being formed at this site. Their location in the first 4 cm. of the duct of Wirsung is significant for two reasons. First, biliary obstruction may supervene because of proximity to the common duct. Secondly, a stone here allows the escape of pancreatic juice via the duct of Santorini if that duct opens into the duodenum. This in turn might prevent marked atrophy of the body and tail with resultant steatorrhea.

Two different patterns of calculous involvement were observed: first, that in which the main ducts were involved, and secondly, that in which only small ducts and acini were concerned. In the first group are the cases with solitary stones in one of the ducts with a variable degree of dilatation of the ducts and atrophy of the parenchyma distal to the calculus. The main duct may contain a large stone near the

duodenum and, back of it, great numbers of smaller calculi may be present in the ducts and/or acini. For some pancreases many calculi were described in the large ducts without mention of the size of the stone nearest the duodenum.

In the second group the large ducts were free of calculi. In the material studied since 1946, we have always been able to dissect the minute calculi from small spaces which microscopically were lined with epithelium. These spaces are evidently ductal. In the material collected previously, several pancreases were described as having only multiple areas of calcification in the parenchyma, with freedom of the large ducts from involvement. Although we shall describe microcalculi in the acini, we have not observed such involvement except in the presence of grossly detectable calculi in the small ducts.

In one patient there was a ductal carcinoma containing tiny calculi in the tail of the pancreas. Distal to the tumor the duct of Wirsung was dilated to a cystic cavity, and connected with this were smaller dilated ducts containing calculi. Calcification of fibrous tissue did occur in the walls of old cysts where the connective tissue had undergone hyalinization. Diffuse calcification of the interstitial tissue was not proved in this series. Apparently the calcium salts were always precipitated in the outflow tract of the pancreatic juice.

Microscopic Observations. The abnormalities observed microscopically were similar to those described for chronic pancreatitis. The differences were in degree of severity. Fibrosis, dilatation of ducts, and atrophy of the parenchyma were more outstanding. In some, fibrosis exceeded that seen in chronic pancreatitis alone. The wide areas of proliferation of edematous connective tissue formed a pattern peculiar to this disease (Fig. 2). A similar change was illustrated in a report by Baggenstoss.[8] Comparatively, the widely dilated ducts with great atrophy of the parenchyma were different also from those ordinarily seen in chronic pancreatitis. In part of the pancreases, however, no differences could be observed between chronic pancreatitis with and without stones. Fibrosis of the perilobular type followed the usual pattern.

Islet destruction, as well as acinar atrophy, was noticeable. The destruction of islets may parallel that of the acini. Pseudocyst formation occurred in a greater percentage (26.9 per cent) than in chronic pancreatitis (9.6 per cent). The relationship of the pseudocysts to the ducts and parenchyma was notable (Fig. 3). Even after pseudocysts had formed, repeated insults might change their character. Necrosis

of their walls with hemorrhage was common; even clots formed in some. No connection with ducts or acini could be seen for some of the pseudocysts.

Of most interest was the appearance of small concretions in the ducts. Two types were seen. The larger aggregates often were laid down in masses of inspissated débris (Fig. 4) or even in the epithelium lining the ducts (Fig. 5). Sometimes intra-epithelial calcification involved areas of squamous metaplasia. That much of the débris was desquamated epithelium was shown by ghost-like remnants exhibiting a columnar arrangement (Figs. 6 and 7). Included in the mixture there was probably protein from the acinar secretion and mucoprotein from the epithelium of the ducts. A second type of precipitate was seen once. It consisted of tiny crystals of calcium widely dispersed in débris (Fig. 8).

Frequently the acini contained microcalculi (Fig. 9). In most of these the epithelium was not discernible; in some, portions at least still remained. It is possible that necrotic epithelium may have formed the basis for calcium precipitation. On the contrary, early post-mortem change may have destroyed the epithelium around the calculi. We are attempting to collect enough fresh material to settle this point.

The duct carcinoma with calculi noted grossly failed to reveal minute calculi on microscopic examination. We have no way of knowing which came first, the carcinoma or the stones. Areas of calculous formation may be limited to the tail. It would not be surprising to find carcinoma arising in ducts which are the site of such remarkable epithelial hyperplasia as is seen in chronic pancreatitis. In several instances it was necessary to study such areas carefully before being certain that malignant change had not occurred.

Chronic inflammation, exemplified especially by round cell infiltration, was present in all cases except those with extreme atrophy and in two with solitary calculi. The perineural round cell infiltration emphasized by Comfort *et al.*[1] was quite striking in several examples.

Acute pancreatic necrosis was noted in 5 cases and was severe enough to contribute to the cause of death in 3.

Lüdin[4] in Basel made post-mortem roentgenograms of 542 pancreases. They were then dissected. He demonstrated calculi in 28, or 5 per cent. We examined 200 unselected pancreases of adults, first by dissection of the duct system and then by roentgenograms. We failed to find any calculi. Tiny areas of increased density noted on some of the plates proved to be calcium in small arteries, the seat of sclerosis.

Thorough dissection of the pancreas revealed calculi in all cases diagnosed roentgenographically during the past 2 years. Undoubtedly, in time we would have found some stones by x-ray examination that we had missed by dissection, but the project did not seem worth pursuing further.

Pathogenesis

We wish to present a possible explanation for the precipitation of calcium salts in the ducts of the pancreas. The normal pancreatic juice is formed at the rate of 2000 to 3000 cc. daily and the rate of its production is more constant than originally thought.[5] The quantity, pH, and enzyme content vary with the stimulating agent. Secretin stimulation following meals produces a free flow of highly alkaline juice poor in enzymes. Vagal or pilocarpine-stimulated juice is smaller in quantity, less alkaline but rich in enzymes. In some animals, the latter type of stimulation can exhaust the secretory granules of the acinar cells without forming enough juice to enter the duodenum. Secretin stimulation occurs chiefly after meals when the acid stomach contents enter the small intestine. This type of stimulation results in flushing out the pancreatic duct system, carrying the needed enzymes into the intestine.

The problem of predicting the nature or extent of precipitation of an insoluble salt in a complex solution such as pancreatic juice presents obvious difficulties. It is, however, possible to calculate whether solubility products of insoluble salts are exceeded, and whether precipitation may be expected to occur.

There is general agreement[6-9] that the total ionic concentration of pancreatic juice is about the same as that of serum, and that the relative proportion of chloride and bicarbonate varies with the secretory activity of the gland. In general, the concentration of bicarbonate is considerably higher than that of serum, and increases with the amount of pancreatic juice formed. In dogs, at least, the bicarbonate may increase at the expense of almost all of the chloride ion,[8] and in man the bicarbonate may reach values of at least 130 mM/l.[9] With the increase in bicarbonate there is, of course, an increase in pH. In the dog, values are commonly from 8.0 to 8.3.[7,8] Except under the influence of mecholyl or secretin, the pH in man usually does not appear to be consistently above 7.5.[9] In measurements on man, of course, some opportunity for neutralization of pancreatic juice by mucoproteins and by contact with the intestinal mucosa is afforded. We may assume, at any rate, that a pH of 8.0 and a bicarbonate concentration

of 100 mM/l are not uncommonly reached. From the second ionization constant of carbonic acid, determined at the ionic strength of pancreatic juice,[10] there would be 1.57 mM/l of carbonate present for each 100 mM/l of bicarbonate at pH 8.0 (0.99 at pH 7.8, and 2.48 at pH 8.2).

The calcium ion concentration found by Ball[11] in the pancreatic juice of dogs was generally 1.0 to 1.5 mM/l. This would correspond roughly to the diffusible calcium of serum and would appear to be a reasonable value. The concentration of phosphate also was found to be somewhat variable, but generally between 0.3 and 0.6 mM/l.[11] Although reliable values for human pancreatic juice under a variety of conditions would be desirable, we will assume that at least 1.0 mM/l of calcium and 0.3 mM/l of phosphate will be found in human pancreatic juice.

At pH 8.0, then, the product of the ionic concentrations of calcium and carbonate may be taken to be $(1 \times 10^{-3})(1.6 \times 10^{-3}) = 1.6 \times 10^{-6}$. The solubility product of $CaCO_3$ at 38° C. and an ionic strength of 0.156 has been found to be between 4 and 5×10^{-8}.[10] Thus, under the above conditions, the solubility product is exceeded in pancreatic juice. If it can be assumed that the pH is determined by the carbon dioxide tension and the bicarbonate concentration, or by some entirely different buffer system, precipitated carbonate would be continually replaced from bicarbonate without any significant change in the bicarbonate concentration or the pH. Precipitation of calcium carbonate could then continue until the solubility product was no longer exceeded. This would correspond to a situation in which $(Ca^{++})(CO_3^{--}) = 5 \times 10^{-8}$, and since (CO_3^{--}) is assumed to be fixed at 1.6×10^{-3}, $(Ca^{++}) = \dfrac{5 \times 10^{-8}}{1.6 \times 10^{-3}} = 3 \times 10^{-5}$. If precipitation took place until equilibrium was reached, 1 — 0.03 mM/l or 0.97 mM/l of Ca^{++} would be precipitated as calcium carbonate. Essentially all of the calcium could be removed in this way, and about 0.1 gm. of $CaCO_3$ could be precipitated from each liter of pancreatic juice. It may be pointed out that, according to this view, increasing the bicarbonate concentration above 100 mM/l, or the pH above 8 would not materially increase the maximum amount of calcium carbonate which could be precipitated, but would increase the initial degree of supersaturation and might promote the initiation of precipitation.

The problem with respect to the probability of precipitation of calcium phosphate from pancreatic juice must be much the same as that of precipitation from serum. If we consider, first, the case of $CaHPO_4$,

the HPO_4^{--} concentration can be calculated from the total phosphate concentration and the second ionization constant of phosphoric acid. At pH 8.0, over 90 per cent of the phosphate will be in the form of HPO_4^{--}, and $(Ca^{++}) (HPO_4^{--})$ will be in the neighborhood of 3×10^{-7}. This is less than the solubility product of 2.5×10^{-6},[12] and precipitation could not take place. For $Ca_3 (PO_4)_2$, however, the solubility product is exceeded. The calculations would be essentially the same as those for serum,[13] except that at pH 8 an even larger proportion of the total phosphate is in the form of PO_4^{---}. If precipitation were to depend, as postulated in the case of bone formation,[13] upon the initial precipitation of $CaHPO_4$ and subsequent modification of the composition of the precipitate to give $Ca_3 (PO_4)_2$, precipitation of calcium phosphate in pancreatic juice would be less likely than precipitation of calcium carbonate. It is well known that calcium carbonate is actually the principal component of pancreatic calculi. This we have found true of the stones we have subjected to analysis.

With these basic factors in mind, what further can be postulated in regard to the formation of calculi? In a normal duct system any small amounts of precipitate formed would flow freely into the duodenum. Occasionally though, as seen in this series, a solitary stone is observed near the ampulla of Vater. Such stones may have started to form farther back in the duct system and lodged near the ampulla, to continue to grow. In symptomless patients without microscopic evidence of chronic pancreatitis some local disturbance in one of the lobules may have started precipitation, or it may be of spontaneous occurrence in the larger ducts. This must be an unusual type, however, for most of the calculi are multiple and often occur following attacks of pancreatitis. In these circumstances, there are the added effects of stasis and inflammation. Dilated ducts filled with stagnant juice would give more opportunity for precipitation. The protein débris in the ducts sometimes seems to act as a nidus for calculous formation. Inflammatory exudate did not seem to be a factor in the precipitation of calcium in the tissues we have studied. The evidence that stasis is important is seen in those patients who have a solitary stone diagnosed by the roentgenogram and in whom, a few months later, many stones are noted back of the first one. This gross pattern was noted twice at necropsy.

The examples of extreme calcification of the pancreas are more difficult to explain. As acinar atrophy becomes advanced the total volume of secretions must fall considerably. Whether enough Ca^{++} and

CO_3^{--} would be secreted to account for the amount of calcification we are not prepared to say. One must consider the possibility of calcium salts being laid down in necrotic acinar epithelium. As noted in Figure 9, acinar calcification occurs, but the fate of the epithelium is difficult to determine.

Since phosphatase has not been observed in acinar cells,[14,15] it need not be considered in the pathogenesis of calculi.

Discussion

From the etiologic standpoint it is difficult to relate either clinically or pathologically all of the pancreatic stones in this series to previous attacks of pancreatitis. This is especially true of the symptomless solitary calculi. Back of the point of obstruction, only dilatation of the ducts and atrophy of the parenchyma were noted. No evidence of chronic inflammation was seen. It must be considered, though, that a subclinical attack of pancreatitis or one forgotten by the patient may have occurred. Opie[16] and Friedreich[17] have commented upon the fact that pancreatic calculi were often symptomless. In a majority of the patients under consideration, a history of excessive use of alcohol and attacks of upper abdominal pain point toward pancreatitis as an etiologic factor. The evidence is not so strong as when the patients have had repeated x-rays and calculi are known to have appeared after bouts of pancreatitis, as described by Comfort *et al.*[1]

The relationship of alcohol to repeated attacks of pancreatitis and the formation of calculi is most striking. It is strange that no attempts have been made to elucidate this connection by animal experimentation. Some of the possibilities worth consideration include: action of alcohol on secretin formation; stimulating effect of blood alcohol on the pancreatic secretion; and the possibility of excretion of alcohol in the pancreatic juice.

The excessive deposit of fat in the liver in patients with pancreatic stones has been noted by Comfort *et al.*[1] Its presence has been explained by lack of lipocaic.[18] We found excess liver fat in 17 (or 65.4 per cent). It was associated with alcoholism and/or cirrhosis in 15, with diabetes in one, and no history was obtainable in the 16th patient. The frequent association of a fatty liver with alcoholism and diabetes is universally recognized. It might be pointed out that failure of the external pancreatic secretion in patients with lithiasis leading to creatorrhea and lack of sufficient absorption of such amino acids as methionine could contribute to the formation of fatty livers.

The association of pancreatic stones with pulmonary tuberculosis has been mentioned repeatedly in the literature. It occurred five times in this group. In three patients, tuberculosis was the primary cause of death. Two patients were diabetic and two gave a history of chronic alcoholism. Both conditions often leave their victims more susceptible to tuberculosis.

From the clinical standpoint, the presence of pancreatic stones should be suspected in patients with diabetes mellitus, chronic alcoholism and cirrhosis, repeated attacks of upper abdominal pain (especially if pancreatitis is suspected), steatorrhea, and unexplained weight loss.

The occurrence of calculi of various sizes along the outflow tract of the pancreatic juice is in accord with the theory that precipitation is due primarily to the supersaturation of pancreatic juice with calcium carbonate. Some factors must operate to prevent the formation of stones, else they would occur more frequently, especially in patients with chronic pancreatitis and dilated ducts in which much protein débris indicative of stasis is often seen. It may be that small calculi are formed in the ducts more frequently than we realize, but pass into the duodenum.

In addition to supersaturation of the pancreatic juice with calcium carbonate, the major rôle of pancreatitis is recognized. Whether these have any direct influence on the formation of calculi is hard to determine. Inflammatory débris and cyst-like dilatation of ducts presumably may initiate the precipitation of calcium carbonate during an acute attack. But it is more plausible to consider that stasis secondary to chronic inflammation is the more important. There is no reason to assume that there would be a change in the pancreatic juice toward the alkaline side in chronic disease. If alcohol acts on the pancreas through a secretin mechanism, excessive stimulation of the formation of alkaline juice may be important.

That calcification of the interstitial tissue may occur we do not deny, but we could find no appreciable evidence of it either in the 62 patients with chronic pancreatitis or in the 26 with stones.

We have never seen calcification of the adipose tissue in and around the pancreas. Even in patients known to have had acute pancreatitis, such lesions have not been seen at necropsy or in surgical material. The fate of the calcium soaps precipitated in the areas of fat necrosis which are seen in acute pancreatitis has been a subject of controversy. Klotz[19] postulated that precipitation of calcium soaps precedes pathologic calcification. The evidence has been discussed by Barr.[20] The experiments

of Wells and Mitchell[21] demonstrating that injected calcium soaps are absorbed, and not converted to inorganic calcium salts, are most convincing. Our studies give no indication that the calcium soaps found around the pancreas in areas of fat necrosis are sites of subsequent calcification. The formation of inorganic calcium deposits where calcium soaps have been deposited would require some mechanism for the rapid removal of fatty acids, leaving a high local concentration of calcium ions. Such a mechanism is not known outside the cell, in which oxidation might occur. It would seem more reasonable to assume that calcium soaps are removed by a gradual process of solution without a local increase in calcium concentration.

It is difficult to explain the difference in incidence of calculi found in post-mortem roentgenograms in Basel[4] and in Los Angeles. This work should be repeated in other parts of the globe. In a series comparable to ours, Simmonds[22] noted in Germany an incidence of 19 in 36,004 necropsies.

Summary

Twenty-six cases of pancreatic lithiasis were found in a series of 36,000 autopsies. Ten of these were discovered in the last 3,000 autopsies.

A history of alcoholism was obtained in 14 (53.8 per cent) and diabetes was a complication in 8 (30.7 per cent).

The deposition of calcium salts was noted only in the outflow tract of the pancreatic juice.

Under normal conditions the pancreatic juice is supersaturated with $CaCO_3$, for the ion concentration product will reach values of at least 1×10^{-6}, whereas the solubility product is about 5×10^{-8}. The precipitation of $CaCO_3$ to form calculi in the ducts of the pancreas can thus be explained.

Other factors affecting the ducts, such as stasis, inflammation and accumulation of protein débris, must be considered in the pathogenesis of calculi.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

Plate 7

Fig. 1. Diagramatic representation of location of stones in main pancreatic ducts. Pancreas approximately one-half normal size.

Fig. 2. Extensive replacement fibrosis of pancreatic lobules, with chronic inflammation and edema. Hematoxylin and eosin stain. × 70.

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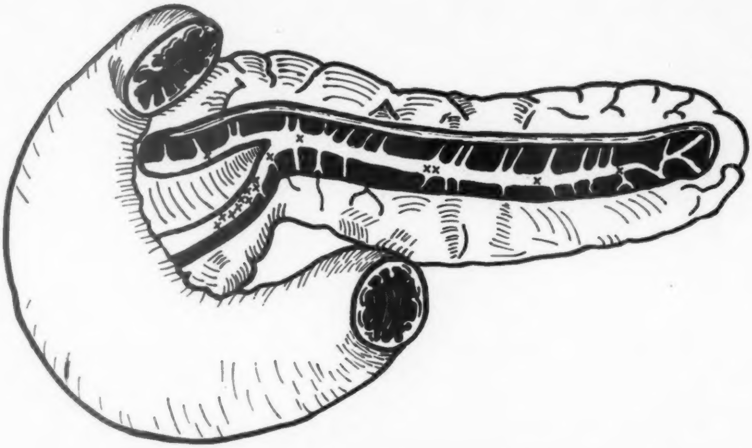

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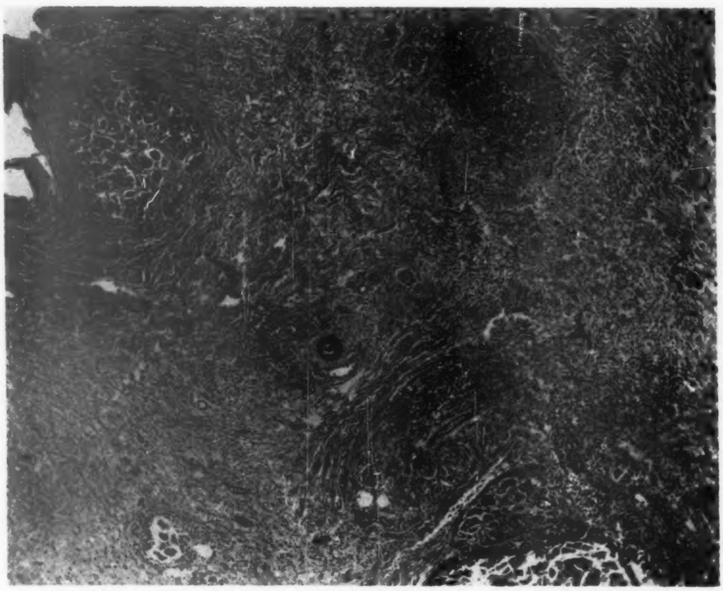

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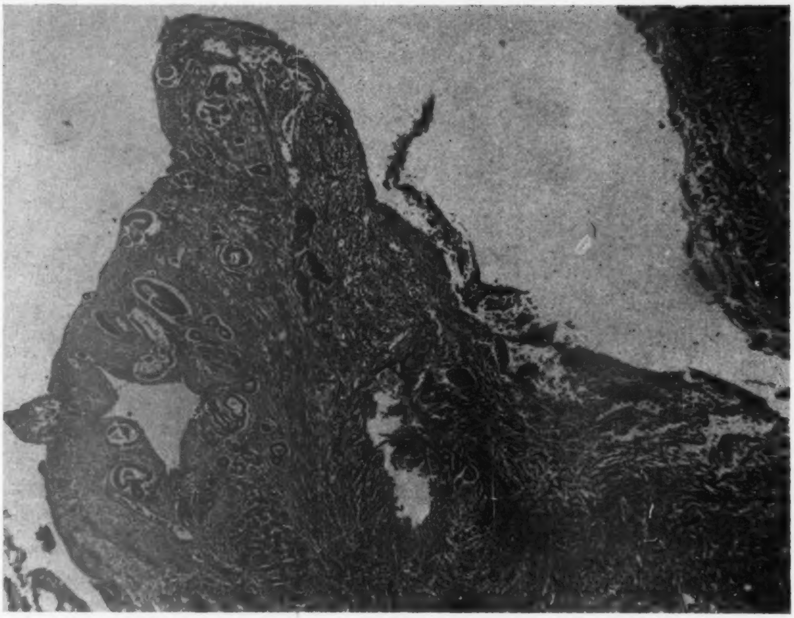
PLATE 8

FIG. 3. Lining of pseudo-cyst with connecting ducts. Some necrotic tissue tags are still attached to lining. Hematoxylin and eosin stain. × 70.

FIG. 4. Microcalculi precipitated in masses of protein débris. Hematoxylin and eosin stain. × 175.

FIG. 5. Multiple calcific masses in ducts and also within the epithelial lining cells. Hematoxylin and eosin stain. × 150.



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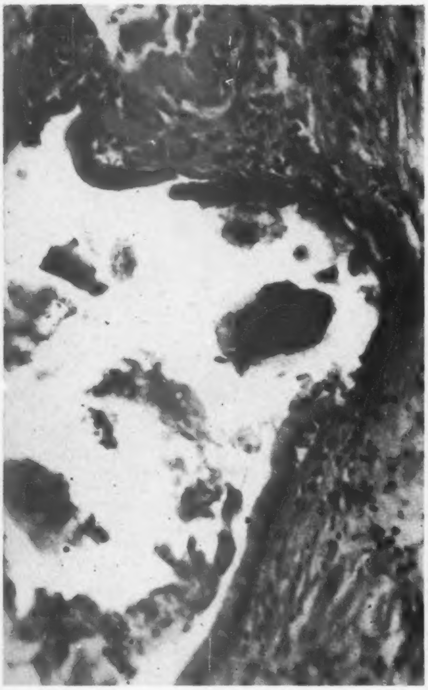

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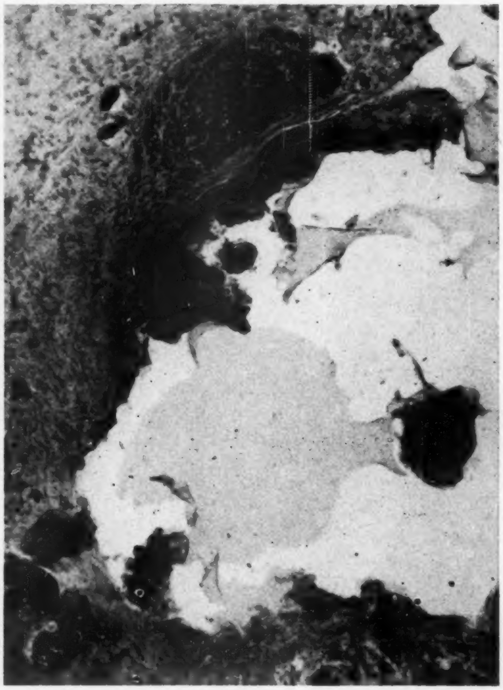

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PLATE 9

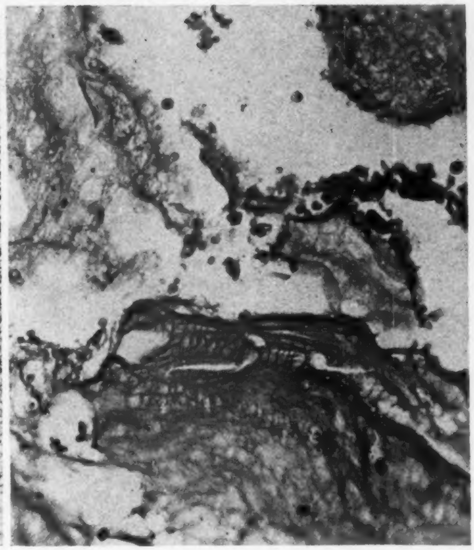
FIG. 6. Dilated duct filled with conglomerate mass of protein. Hematoxylin and eosin stain. $\times$ 55.

FIG. 7. Higher magnification of protein débris shown in Figure 6. Of note are the remnants of columnar epithelium. Hematoxylin and eosin stain. $\times$ 145.

FIG. 8. Fine crystalline calcium precipitate widely dispersed in protein débris in small duct. Severe chronic pancreatitis and atrophy. Hematoxylin and eosin stain. $\times$ 55. Polarized light photomicrograph.

FIG. 9. Spherical intra-acinar masses of calcium salts. These appear to be precipitated in necrotic acinar epithelium. Hematoxylin and eosin stain. $\times$ 205.

6



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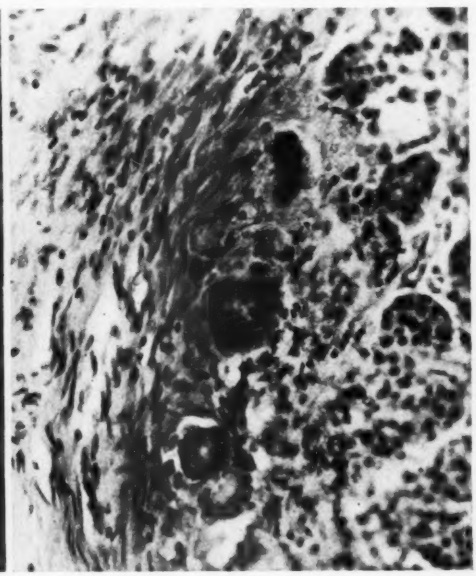

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Chronic Pancreatitis and Lithiasis, II

PROLAPSE OF REDUNDANT GASTRIC MUCOSA THROUGH THE PYLORIC CANAL INTO THE DUODENUM*

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In the medical literature to 1947, 37 cases of prolapse of the gastric mucosa into the duodenum were found recorded, and 16 additional cases diagnosed roentgenologically were reported by Manning and Highsmith.[1] It was believed that prolapse of redundant gastric mucosa through the pyloric canal into the duodenum is more common than is generally recognized, although not necessarily of clinical significance. In a survey of upper gastro-intestinal roentgenograms from 7,317 patients examined in the X-ray Department of Watts Hospital, 91 cases (1.24 per cent) were found with roentgenologic features considered characteristic of prolapse of the gastric mucosa. In the same group of patients, duodenal ulcer was diagnosed in 804 (10.9 per cent), gastric ulcer in 187 (2.55 per cent), and diverticulum of the duodenum in 88 (1.20 per cent). Roentgenologic findings suggestive of hypertrophic gastritis were noted in 349 patients (4.77 per cent).

The symptomatology and roentgenologic characteristics of prolapse of the gastric mucosa have been discussed in the earlier report.[1] Upper abdominal or epigastric discomfort, gaseous distress, burning pain, nausea, vomiting, hematemesis, melena, anemia, and "ball valve" type of intermittent pain and obstruction were symptoms most commonly encountered. Roentgenograms of the upper gastro-intestinal tract characteristically show a central, circular or irregularly circular, umbrella or mushroom shaped filling defect at the base of the duodenal bulb, which is most frequently noted in the horizontal and right oblique horizontal positions. Heavy mucosal folds, especially in the antrum, often with antral spasm, may be seen, and may have an irregular arrangement rather than the usual horizontal and vertical pattern. The pyloric channel may be elongated and narrowed.

Pathologic studies as previously reported have been confined largely to examination of tissue removed at surgical operation, inasmuch as many pathologists have failed to search for the condition at necropsy. When the stomach is opened during the usual post-mortem examination, there is a tendency for the redundant mucosa to fall into a more normal position and the redundancy is overlooked unless searched for and tested by manipulating the loose mucosa with forceps. Similarly, surgical exploration often fails to reveal prolapse of the gastric mucosa on

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external palpation of the stomach and duodenum, and the lesion may be missed unless the mucosa is actually inspected. In the cases which we have studied post mortem, however, the redundant mucosa usually was quite apparent when the stomach and duodenum were opened. There is a certain looseness of the mucosa in the antral region normally, but in patients with marked prolapse of the gastric mucosa, the mucosal looseness is exaggerated and the redundant folds either lie in the pyloric canal or can easily be drawn through it for variable distances into the duodenum.

Opportunity to study this condition at post-mortem examination has been possible in 6 patients, in 4 of whom a roentgenologic diagnosis of prolapse of the gastric mucosa was made prior to death. In the other 2 cases, roentgenologic studies of the upper gastro-intestinal tract had not been made in one and the prolapse was not recognized roentgenologically in the other. In no case did prolapse of the gastric mucosa have any direct responsibility for death, although gastric hemorrhage contributed to death in case 3, and a perforated gastric ulcer caused death in case 4.

Report of Cases

Case 1

S. S. S., a white woman, 66 years of age, had had multiple gastro-intestinal complaints of 30 years' duration and had been admitted to the hospital for numerous complaints. The details of her clinical history have been reported previously.[1] In 1940 she developed a medullary carcinoma of the left breast for which a radical mastectomy was performed, followed by two courses of irradiation therapy. In August, 1946, gastro-intestinal roentgenograms revealed a small esophageal hiatus hernia and considerable exaggeration of the mucosal folds of the stomach, particularly in the antrum. There was marked antral spasm and a small irregular filling defect was noted at the base of the duodenal cap suggesting early prolapse of the gastric mucosa into the duodenum. Re-examination of the gastro-intestinal tract in June, 1947, again revealed marked exaggeration of the mucosal folds in the antrum and, on serial films, a considerable extension of the redundant mucosal folds through the pylorus into the duodenum could be seen to occur in antral systole (Fig. 1). Subsequently, the patient developed metastases of the carcinoma of the breast to the right lung and expired in January, 1948.

An autopsy was performed 7 hours after death.

Gross Examination. At gross examination, the wall of the stomach was thickened and the mucosal folds were prominent. In the antrum, the mucosa was redundant and was prolapsed through the pyloric sphincter for a distance of about 8 mm. (Fig. 2). The redundant folds could be drawn readily into the duodenum. The submucosa was very loose, permitting the mucosa to slip freely over the muscularis. The entire circumference of the mucosa was involved in the prolapse. The

mucosa in this region was congested and showed several small superficial ulcerations. The rugae in the pyloric region and antrum were oriented transversely.

Microscopic Examination. Microscopically, the gastric mucosa was heavily infiltrated with lymphocytes and plasma cells. This chronic inflammatory process was most marked in the prolapsed portion. The muscularis mucosae appeared normal except for lymphocytic infiltration in places. The pyloric sphincter was a prominent muscular bundle projecting into the base of the prolapsed fold. The connective tissue between the muscularis mucosae and the muscularis was quite loose and rather widely separated, undoubtedly accounting for the abnormal mobility of the mucosa (Figs. 3 and 4).

Case 2

E. S. R., a white retired farmer, 78 years old, had had a long series of hospital admissions, beginning in 1930, for chronic prostatitis, bronchial asthma, chronic bronchitis, emphysema, chronic sinusitis, hypertensive vascular disease, generalized arteriosclerosis, arteriosclerotic heart disease, cerebral thrombosis, chronic cholecystitis with obstructive jaundice, and anterior myocardial infarction. In January, 1947, jaundice recurred with abdominal pain, nausea and vomiting. A diagnosis of acute cholecystitis was made, but surgery was deferred upon subsidence of the jaundice because of his poor physical condition. He was readmitted in February, 1948, for an attack of upper abdominal pain and stupor occurring on the day of admission. Physical examination revealed mental confusion and apathy, immobile facies, "cog wheel" rigidity of the extremities, general hyperreflexia, emphysema, râles at the lung bases, enlargement of the heart with poor heart sounds, marked abdominal distention and tympanites, and generalized abdominal tenderness most marked in the right upper quadrant. Roentgenograms of the chest revealed approximately 20 per cent cardiac enlargement with pulmonary congestion. Electrocardiograms were consistent with healed anterior myocardial infarction. There was moderate nitrogen retention, and while under observation the patient developed clinical jaundice. His condition deteriorated progressively and he expired in February, 1948.

Roentgenograms of the upper gastro-intestinal tract up to January, 1947, were reported as normal, and prolapse of the gastric mucosa into the duodenum was not recognized prior to death. His chronic gastro-intestinal symptoms had been attributed to the associated diseases, such as chronic cholecystitis.

An autopsy was performed 3 hours after death.

Gross Examination. The stomach was small and practically empty. Its mucosa was thickened. The rugae were prominent and arranged longitudinally. The mucosa in the region of the pylorus was redundant and the pyloric muscle moderately hypertrophied so that the pyloric channel was unusually small. The mucosa projected for about 6 mm. beyond the sphincter (Fig. 7) and could be easily pulled into the duodenum for a distance of 20 mm. The submucosa was loose so that the mucosa slipped freely over the muscularis.

Microscopic Examination. Microscopically, chronic inflammation was present in the prolapsed mucosa and the muscularis mucosae. The redundant portion of the mucosa was thrown into several small folds and one heavy fold in which the muscularis mucosae was unusually prominent, filling most of the central portion of the redundant fold. The submucosa was loose, and there was considerable separation of the mucosa and muscularis. The pyloric sphincter was a thickened muscular bundle projecting for a short distance into the base of the prolapsed fold (Fig. 8).

Case 3

S. L. R., a white man, 47 years of age, was admitted to the hospital in April, 1948, with a history of hypertension of 9 years' duration, forcing his retirement from business for the previous 2½ years. He had improved temporarily on a rice diet regimen, but for 2 months prior to admission had begun to go downhill with increasing weakness, anorexia, vomiting, insomnia, restlessness, intermittent severe frontal headache, staggering gait, dyspnea on exertion, chronic cough, frequency of urination, and nocturia. Because of gastro-intestinal symptoms, he had roentgenograms of the upper gastro-intestinal tract 2 weeks prior to admission which revealed the stomach to be high and hypertonic, with a concave deformity at the base of the duodenal cap consistent with some prolapse of the gastric mucosa into the base of the cap. There was a small diverticulum of the second portion of the duodenum. A roentgenologic diagnosis of prolapse of the gastric mucosa was made (Fig. 5).

Admission examination revealed a chronically ill, nervous, emotionally unstable man with uriniferous breath. The blood pressure was 230/160 mm. of Hg, the heart was enlarged with moderate tachycardia, and there was general hyperreflexia. Laboratory studies revealed low fixed urinary specific gravity, moderate albuminuria, cylindruria, mild normochromic anemia, negative Kahn and Mazzini tests, and nitrogen retention with blood urea of 128 mg. per 100 cc. and creatinine of 5.8 mg. per 100 cc. The CO_2-combining power was 46 volumes per cent and phenolsulfonphthalein excretion was less than 5 per cent in 2 hours.

On the sixth hospital day, the patient suddenly vomited about 500 cc. of old clotted blood and went into shock. His blood pressure was restored with plasma and whole blood, but his general condition remained poor with Cheyne-Stokes respiration and increasing nitrogen retention. He expired of respiratory failure on May 2, 1948.

An autopsy was performed 2 hours after death.

Gross Examination. The gastric mucosa at the pylorus was prolapsed into the duodenum in the form of the two tongue-like projections. One of these, located on the posterior aspect of the pyloric ring, arose from a broad base about 10 mm. proximal to the pyloric sphincter and projected as a flat polypoid structure for about 15 mm. beyond the sphincter. The other prolapsed fold was on the anterior aspect and extended about 15 mm. into the duodenum (Fig. 9). The gastric rugae were oriented more or less transversely at the pylorus.

Microscopic Examination. Microscopically, moderate chronic inflammation was present in the gastric mucosa, including the prolapsed

portion. The side of the mucosal projection facing the duodenum contained submucosal glands of Brunner. The muscularis mucosae was prominent, forming part of the core of the mucosal projection. The submucosa was loose and the pyloric musculature appeared moderately hypertrophied (Fig. 10).

Case 4

D. P. C., a white bank executive, 58 years old, was hospitalized in August, 1947, for "weak spells." At that time he complained of gaseous indigestion occurring 10 to 60 minutes after meals, epigastric and substernal fullness, and bloating relieved by belching, of several years' duration. Physical examination was normal. Roentgenograms of the upper gastro-intestinal tract revealed considerable irregularity of the mucosal folds of the stomach and spasm of the antrum. Marked pylorospasm was present. A filling defect in the base of the duodenal cap was noted and considered consistent with prolapse of the gastric mucosa. A clinical diagnosis of hypertrophic antral gastritis and prolapsed gastric mucosa was made. On November 12, 1947, he had four carious teeth extracted. About December 18, 1947, he began to have nocturnal chills and fever with generalized muscular and arthralgic pains. He was hospitalized on December 26, 1948, and remained in the hospital until 5 weeks prior to his death on July 27, 1948. During this period of approximately 7 months he ran an irregularly febrile course with muscle tenderness, development of severe polyneuritis involving the distal parts of the extremities and other areas with anesthesias, paresthesias, trophic skin changes, and localized muscular weakness. On March 7, biopsy of inguinal nodes revealed changes in the small arteries of the nodes suggesting periarteritis nodosa. In spite of varied medical therapy, his course was slowly downhill with intermittent pyuria, hematuria, albuminuria, progressive myocardial damage with cardiac enlargement, increasing tachycardia, gallop rhythm, electrocardiographic changes and congestive failure with pulmonary and hepatic congestion. Increasing mental apathy and drowsiness were noted. A clinical diagnosis of periarteritis nodosa was made. He expired at home on July 27, 1948, death being attributed by his physician to terminal pneumonia.

Repeated gastro-intestinal roentgenograms during his last hospitalization confirmed the previous finding of a marked umbrella deformity at the base of the duodenal cap, characteristic of prolapse of the gastric mucosa into the duodenum (Fig. 6).

An autopsy was performed 9 hours after death.

Gross Examination. The entire gastric mucosa was thickened, edematous and congested, and the mucosal folds were prominent. The mucosa was redundant in the pyloric region and projected into the duodenum for a distance of about 5 mm. The gastric rugae were not prominent at the pylorus and were transverse in arrangement. There seemed to be little looseness of the submucosa, for in this specimen the mucosa could not be moved over the muscularis as easily as in the others (Fig. 12). On the anterior surface of the stomach near the greater curvature and 12 cm. proximal to the pylorus was a large peptic ulcer which had perforated.

Microscopic Examination. In the pylorus moderate chronic inflam-

mation was present microscopically. The submucosa was inconspicuous and contained very little fat. The core of the prolapsed mucosa was formed of muscularis mucosae and muscularis of the prominent pyloric sphincter with only a little fibrous submucosa between them (Fig. 13).

Case 5

M. T. U., a woman, 67 years old, was hospitalized on the surgical service in October, 1946, complaining of a "growth" in the left popliteal fossa and a chronic cough of 2 years' duration. The popliteal mass was explored and found to be fat and hypertrophy of the external head of the gastrocnemius muscle. Roentgenograms of the chest at this time revealed an area of consolidation extending out from the right hilum into the right upper lobe. She returned approximately 1 year later, in December, 1947, complaining of gaseous distress and slight discomfort in the upper quadrant of 5 to 6 weeks' duration, with anorexia, nausea, vomiting, vertigo, chills and fever of 1 week's duration. Roentgenograms of the chest revealed considerable increase in the size of the consolidated mass extending from the right hilum. The roentgenologic findings suggested bronchogenic carcinoma. Upper gastro-intestinal studies were reported as normal except for a small esophageal hiatus hernia. Bronchoscopic examination failed to demonstrate any disease in the proximal branches of the right bronchus. The patient remained under intermittent hospital observation until May 20, 1948, when she was readmitted because of nausea, vomiting, and postprandial gaseous distress of 1 week's duration. In June, 1948, she was transferred to another hospital where a lobectomy was performed with the operative finding of an adenocarcinoma of the right bronchus. Her condition deteriorated progressively and she was rehospitalized on August 5, 1948, for headache, vomiting, periods of unconsciousness, malnutrition, weakness, irrationality, and finally stupor. She expired on August 23, 1948. The clinical diagnosis at death was adenocarcinoma of a bronchus with metastases to brain. Roentgenograms of the gastro-intestinal tract in the latter periods of hospitalization revealed a small filling defect at the base of the duodenal cap consistent with prolapsed gastric mucosa (Fig. 11).

An autopsy was performed 3 hours after death.

Gross Examination. The stomach was normal grossly except at the pylorus. Here there was a circumferential prolapse of the gastric mucosa into the duodenum for a distance of 8 to 10 mm. A few longitudinal rugae were present in the antrum. The pyloric sphincter was of normal thickness. About 3 cm. distal to the pylorus was a shallow ulcer in the duodenal mucosa measuring 6 by 4 mm. The submucosa was quite loose and the mucosa slipped freely over the muscularis (Figs. 14 and 15). (This specimen was discarded accidentally and no microscopic study was made.)

Case 6

N. W., a white woman, 59 years of age, was admitted to the hospital on August 25, 1948, and expired on September 28, 1948. Her history revealed that she had spent a number of years in England and India where she had had typhoid fever, dengue, tertian malaria, amebic dysentery, and pneumonia. For 15 years she had been known to have diverticulosis and diverticulitis of the colon, with chronic

constipation and intermittent abdominal pain for which she had been treated with dietary regimen. For 3 weeks prior to admission she had noted weight loss, increased constipation, abdominal pain, and fever. Barium enema had revealed a partial obstruction of the colon. Proctosigmoidoscopy indicated a fungating tumor mass obstructing the colon 7 cm. from the anus. A specimen for biopsy from this mass was reported as adenocarcinoma. Exploratory laparotomy on September 20 revealed a lemon-sized mass at the rectosigmoid junction with extensive carcinomatous metastases throughout the peritoneal cavity. On September 28 she passed into shock, had cramping abdominal pain, passed a large amount of blood by rectum, and expired.

An autopsy was performed 3 hours after death.

A roentgenographic gastro-intestinal series was not obtained on this patient and prolapse of the gastric mucosa was never suspected clinically.

Gross Examination. The stomach and duodenum were distended. There was a peculiar tongue-shaped redundancy of the gastric mucosa projecting into the duodenum for a distance of about 10 mm. The muscularis was thin and it was difficult to distinguish the pyloric sphincter grossly. The mucosa appeared atrophied and its rugae were not well developed. There was a cobble-stone appearance (Fig. 16).

Microscopic Examination. Slight chronic inflammation was seen in the prolapsed fold on microscopic examination. The muscularis mucosae was of normal thickness. The submucosa appeared loose and contained very little fat. The muscle of the pyloric sphincter was poorly developed (Fig. 17).

Discussion

An effort was made to correlate the various anatomic features presented by the 6 cases. In all 5 examined microscopically, mild to severe chronic gastritis was present. Grossly and microscopically the gastritis appeared to be of the hypertrophic variety except in case 6 in which atrophic changes predominated in the mucosa. In 4 cases the submucosa was unusually loose, so that the mucosa slid easily over the muscularis. In case 6, the redundant mucosa appeared as a tongue-like, polypoid fold protruding through the pylorus, with no unusual looseness of the submucosa. In case 4, the prolapsed mucosa was a hypertrophied fold forced caudally into the duodenum by the hypertrophied pyloric muscle. The pyloric muscle was thickened and the pyloric channel appeared to be narrowed in 4 cases. The muscularis mucosae was thickened in 3 cases and showed lymphocytic infiltration in one. In 4 cases the entire circumference of the pyloric and prepyloric mucosa was involved in the prolapse. In 2 cases the prolapse was in the form

of a polypoid or tongue-like projection involving only a part of the pyloric circumference. In all cases except one the antral rugae were oriented in an irregular manner, the predominant orientation being transverse in 3 and longitudinal in 2. In case 6 the mucosa was atrophic and presented a cobble-stone appearance with complete loss of rugal folds.

The pathologic study of these cases appeared to support the observations of Eliason and Wright,[2] in 1925, that chronic irritation of the gastric mucosa due to physical, nutritional, functional, chemical, or bacterial causes results in chronic inflammation and hypertrophy of the mucosa, with consequent looseness and redundancy which is increased mechanically by the peristaltic contractions of the stomach and the pressure of the gastric contents as they are forced on to the pylorus. This theory of pathogenesis, with the observation of Rees[3] that hyperperistalsis of the stomach forcing its contents through a narrowed pylorus increases the trauma to which the redundant mucosa is subjected, would appear to have been operative with certainty in 3 cases and possibly in 2 others. In case 6, the mechanism seems to have been somewhat different, although the same factors may have produced the polypoid, tongue-like projection of pre-pyloric mucosa. This elongated fold protruded into the duodenum by virtue of its length and shape rather than by a sliding movement of the mucosa over the muscularis. Golden[4] has observed that the muscularis mucosae normally contracts in antral systole and produces a cephalad tautening of the antral mucosa with a rearrangement of the transverse rugal folds into a more longitudinal pattern, thereby tending to prevent the pressure effects of the gastric contents and the peristaltic contractions which tend to force the antral mucosa into the duodenal cap. That interference with this physiologic mechanism may have occurred in at least 3 of the reported cases is suggested by the finding of hypertrophy of the muscularis mucosae in those 3 cases and inflammatory involvement in a fourth case. The mechanical effects exerted by the hypertrophied mucosa on the muscularis mucosae or by inflammation of the muscularis mucosae interfering with its contractibility possibly interfered with the normal tautening of the mucosa in antral systole, thereby allowing increased redundancy of the mucosa and increasing its susceptibility to peristaltic contractions and the pressure of the gastric contents. In case 6 gravitational force, as well as peristaltic contractions and pressure from gastric contents, would have carried the tongue-like fold into the duodenum.

Superficial ulceration of the prolapsed mucosa occurred in case 1. Gross hemorrhage[5] and slow oozing of blood from ulceration of the prolapsed mucosa,[6,7] "ball valve" obstruction and partial to complete pyloric obstruction,[3,6,7,8] polypoid degeneration,[2,6,7] and malignant proliferation[9] have been noted in previous reports. Hemorrhage from the stomach occurred in case 3, but the point of hemorrhage could not be located at necropsy and hemorrhage could not be attributed to the prolapse. Partial obstruction probably occurred in case 1 but was never severe. Polypoid changes were encountered in 2 cases but malignant proliferation was not noted. In the 6 cases reported, there seemed to be no particular relationship of the prolapse of the gastric mucosa to the conditions which caused death.

Summary

Study of the pathologic findings in 6 patients with prolapse of the gastric mucosa through the pyloric canal into the duodenum indicated that chronic inflammation of the antral mucosa with loosening of the submucosa, allowing the mucosa to slide easily over the muscularis through the pylorus into the duodenum under the action of peristaltic contractions of the antrum and the pressure of the gastric contents, was the probable explanation for prolapse in 4, and possibly in 5 cases. In one case the prolapsed mucosa was a tongue-like polypoid projection of the antral mucosa which prolapsed by virtue of its length and location, rather than as a result of loosening of the mucosa on the muscularis. There was no apparent relationship between gastric mucosal prolapse and the cause of death in these 6 patients.

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DESCRIPTION OF PLATES

Plate 10

Fig. 1. Case 1. Extrusion of the markedly redundant gastric mucosa into the duodenum in antral systole (A) and the position of the antral folds a few minutes before when the antrum was relaxed (B). (Reprinted by permission from *Gastroenterology*, 1948, 10, 657.)

Fig. 2. Case 1. The prolapsed gastric mucosa projects about 8 mm. beyond the pyloric sphincter. Several small ulcerations are visible.

Fig. 3. Case 1. Chronic inflammation of the prolapsed mucosa with loose submucosa and hypertrophied pyloric muscle. Hematoxylin and eosin stain. × 5.5.

Fig. 4. Case 1. There is dense infiltration of the prolapsed gastric mucosa with lymphocytes and plasma cells. Hematoxylin and eosin stain. × 175.

1
A

B

2

3

4

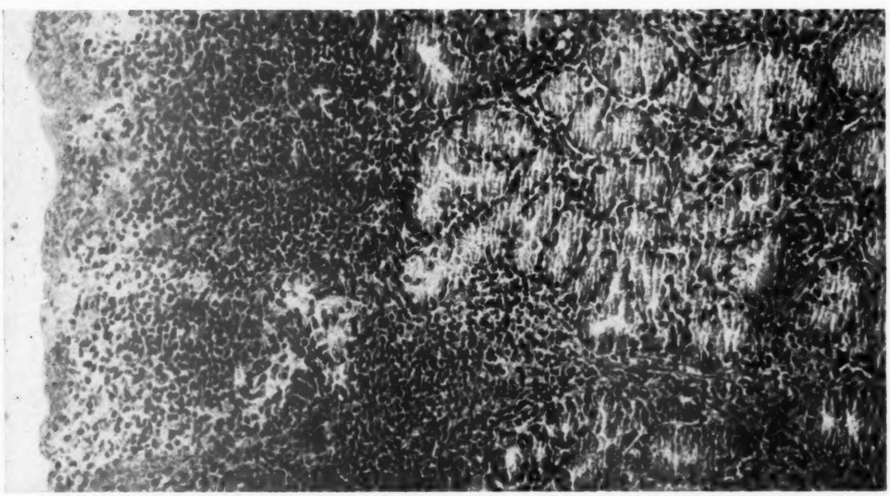

PLATE 11

FIG. 5. Case 3. Umbrella defect at the base of the duodenal cap.

FIG. 6. Case 4. Large mushroom filling defect at the base of the duodenal cap.

FIG. 7. Case 2. The thick longitudinal rugae project about 6 mm. into the duodenum and could be easily pulled 20 mm. into the duodenum.

FIG. 8. Case 2. Thickened redundant mucosa, hypertrophied muscularis mucosae, loosened submucosa, and hypertrophied pyloric muscle. Hematoxylin and eosin stain. $\times$ 4.5.

FIG. 9. Case 3. Two tongue-like projections of gastric mucosa extend into the duodenum. One of these, located on the posterior aspect of the pylorus, arises about 10 mm. proximal to the pyloric sphincter.

FIG. 10. Case 3. Two thickened, loosely attached mucosal folds with hypertrophied muscularis mucosae and pyloric muscle. Hematoxylin and eosin stain. $\times$ 4.5.

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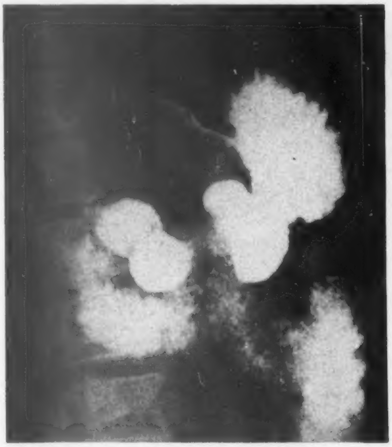

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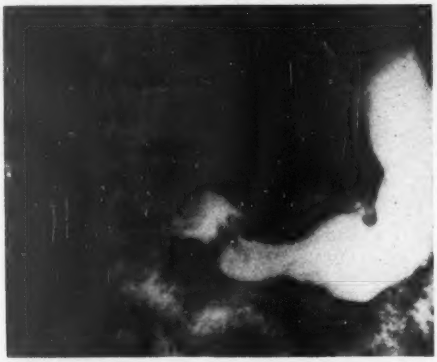
PLATE 12

FIG. 11. Case 5. Small umbrella defect at the base of the duodenal cap.

FIG. 12. Case 4. The circumferential prolapse of the gastric mucosa extends about 5 mm. into the duodenum.

FIG. 13. Case 4. Protruding mucosal fold and hypertrophy of the pyloric muscle. Hematoxylin and eosin stain. × 6.5.

11



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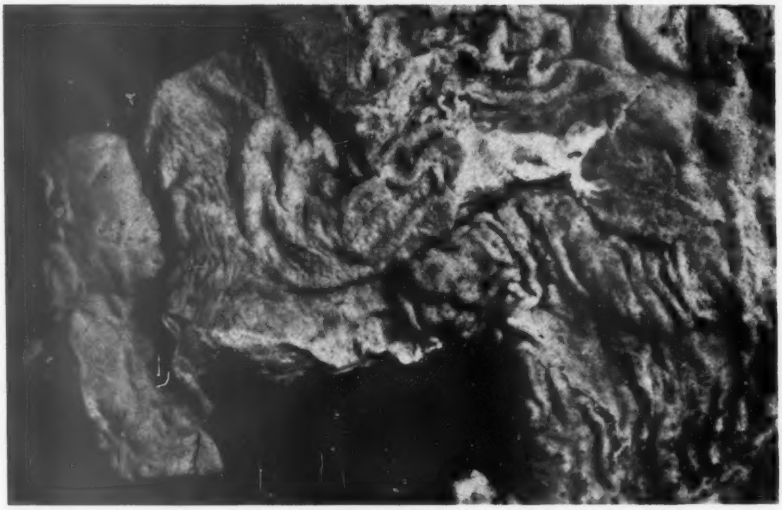

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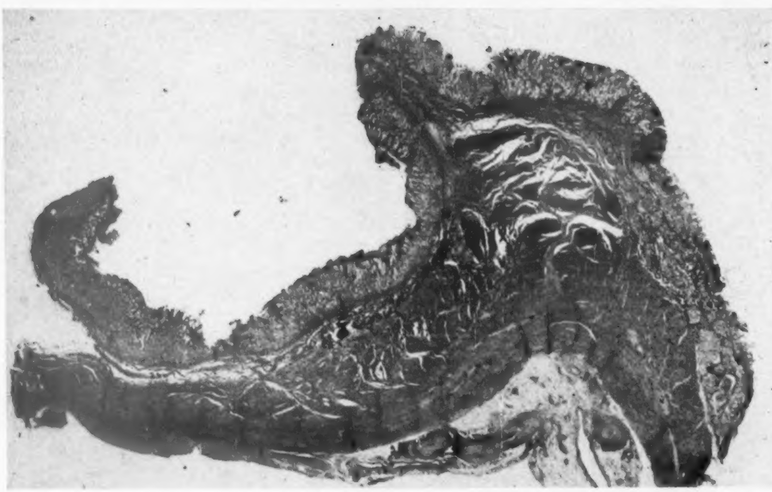

PLATE 13

FIG. 14. Case 5. Circumferential prolapse of the gastric mucosa extending for 8 to 10 mm. into the duodenum. A small ulcer can be seen in the duodenal mucosa about 3 cm. distal to the pylorus.

FIG. 15. Case 5. Same as Figure 14, with prolapsed mucosa reflected back.

FIG. 16. Case 6. Broad tongue-like redundancy of the gastric mucosa projecting into the duodenum, with cobble-stone appearance of the mucosa.

FIG. 17. Case 6. Elongated redundant fold of atrophic prolapsed mucosa with small pyloric muscle. Hematoxylin and eosin stain. $\times$ 6.5.

15

17

Manning and Gunter

Prolapse of Redundant Gastric Mucosa

LYMPHOID TISSUE AND ITS RELATION TO SO-CALLED NORMAL LYMPHOID FOCI AND TO LYMPHOMATOSIS

II. Quantitative Analysis of Lymphoid Areas in the Pancreas of Laboratory and Farm Chickens*

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In the preceding paper of this series[1] the normality of lymphoid areas in the pancreas of the fowl was questioned, and it was shown wherein many of them are definitely abnormal and destructive to adjacent pancreatic tissue. Moreover, no significant histologic difference between those lymphoid areas which are destructive and those which seemingly are not destructive was found. This observation was applicable to all except one case, which had 88.4 per cent of the section involved. At this percentage level the lymphoid tissue showed disorganized growth and very large lymphoid cells not found in the other 193 cases showing lymphoid tissue. No apparent significance could be attached to the encapsulated, focal perivascular, or diffuse types of lesions. The encapsulated focal type appeared to be derived from small plugged blood vessels. Since no qualitative basis seemed evident for separation of microscopically positive and negative cases of lymphomatosis, a quantitative study of lymphoid tissue was made on the same material and is reported in this paper.

A quantitative study of lymphoid tissue for the fowl never has been reported to our knowledge; and with the availability of inbred lines showing different percentages of grossly visible lymphoid tumors, an opportunity is offered to make an analytical study of the lymphoid reactions to lymphomatosis.§

This is not a microscopic study of grossly visible tumors but is a study of the pancreas from birds which survived the experimental period. In 95.6 per cent of the cases the birds were diagnosed grossly as negative for lymphomatosis, and in every case the pancreas was reported to be negative for grossly visible tumors.

In the various inbred lines of chickens used in this study there were

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‡ United States Department of Agriculture, Agricultural Research Administration, Bureau of Animal Industry.

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TABLE I

Summary of G (1945) Population of Laboratory Stock (Females Only)

Selected* for:		R	S	R	S	R	R	S	S	R	S	R	R	S	S	S × R	
Line numbers:		1	2	3	4	5	6	7	9	10	11†	12	13	14	15	9 × 10	Total
Families	*Classes*																
1	T.P.	10	10	9	14	2	10	13	12	11	19	7	10	3	14	12	
	D.W.L.	1	6	8	3	0	0	2	5	4	6	6(1)	4	1	2	3	
	O.D.	6	4	1	3	2	0	3	4	4	7	1	4	1	3	0	
	C.P.T.	3	0	0	8	0	7^3	8	2^1	2^1	5^1	1	2	1	9	9	
2	T.P.	10	9	9	10	1	10	12	25	10	15	4	10	11	9	7	
	D.W.L.	1	6	8	3	0	0	1	12	3	8	3	1	7	7	1	
	O.D.	4	3	1	6	0	1	4	13	1	6	1	3	3	1	1	
	C.P.T.	5	0	0	1	1	5^4	7	0	6	1	0	6	0^1	1	5	
3	T.P.	10	9	14	23	3	10	8	20	9	12	2	11	10	14	16	
	D.W.L.	4	8	3	14(1)	0	3	2	13	1	4	1	3	5	1	9	
	O.D.	4	1	5	7	2	2	2	6	3	6	1	5	5	4	6	
	C.P.T.	2	0	6	3	1	5	3^1	1	5	2	0	3	0	9	1	
4	T.P.	11	18	16	18	1	10	11	26	10	15		10	3	16	26	
	D.W.L.	2	8(1)	11	8	1	1	0	9(1)	2	7		3(1)	3	11	7	
	O.D.	5	10	2	7	0	1	5	11	3	7		3	0	3	2	
	C.P.T.	4	1	3	3	0	2^6	6	6^1	5	1		5	0	2	16^1	
5	T.P.	10	22	17	10	9	13	15		11	15		10	6	20	12	
	D.W.L.	2(1)	10	9	6	2	0	4		4	7		[illegible]	1	17	5(1)	
	O.D.	5	12	4	2	4	3	3		2	8		5	5	3	1	
	C.P.T.	4	0	4	2	3	4^6	8		5	0		4	0	0	7	
6	T.P.		11	13	13	2		12		11	18		12	10	19	21	
	D.W.L.		7	4	6	2		4(1)		1	6		4	4	8(2)	7	
	O.D.		2	1	2	0		3		7	10		7	2	6	4	
	C.P.T.		2	8	5	0		6		3	0^2		1	4	7	10	

7	T.P.		8	8	10	3		15			15						
	D.W.L.		2	3	6	1		5(1)			8						
	O.D.		6	1	2	0		4			5						
	C.P.T.		0	4	2	2		7			2						
8	T.P.			7	10			11									
	D.W.L.			5	6(1)			1									
	O.D.			1	2			3									
	C.P.T.			1	3			7									
9	T.P.			11	17			15									
	D.W.L.			7(1)	5			7(1)									
	O.D.			2	6			4									
	C.P.T.			3	6			5									
10	T.P.							11									
	D.W.L.							4									
	O.D.							2									
	C.P.T.							0^{5}									
Summary for lines	T.P.	51	87	104	125	21	53	123	83	62	109	13	63	43	92	94	1123
	D.W.L.	10(1)	47(1)	58(1)	57(2)	6	4	30(3)	39(1)	15	46	10(1)	16(1)	21	46(2)	32(1)	437(14)
	O.D.	24	38	18	37	8	7	33	34	20	49	3	27	16	20	14	348
	C.P.T.	18	3	29	33	7	23^{19}	57^{6}	9^{2}	26^{1}	11^{3}	1	21	5^{1}	28	48^{1}	319^{33}
Average age at termination		619	601	610	614	571	615	622	617	613	628	603	615	603	601	610	613

* The records on selection within lines for resistance (R) or susceptibility (S) to lymphomatosis have been taken from published data by Waters.[8]

† In line 11 because of the loss of males in 1943 the hens were mated with a male from line 2.[4]

T.P. = Total population in the family after 30 days of age.
D.W.L. = Dead with gross lymphomatous tumors.
O.D. = Other deaths.
C.P.T. = Cases from which pancreas tissue was collected. Exponent is number of survivors from which tissues were not collected.
( ) = Birds which were killed at termination showing lymphoid tumors and from which tissues were taken are indicated by numerals in parentheses; so in these cases the same bird is included under D.W.L. and C.P.T.

deaths associated with other pathologic conditions not ordinarily attributed to the agent or agents producing lymphoid tumors. The question immediately arises: is there a relationship between deaths without grossly visible tumors and deaths with grossly visible tumors?

Other investigators[2,3] have observed that the incidence of mortality associated with lymphoid tumors and mortality from other causes showed fluctuations in similar directions.* In the history of the Laboratory there have been no reports of any infectious disease, other than lymphomatosis, in any population, including those hatched in 1945 upon which this work is based. No internal or external parasites have been observed except a low incidence of coccidia. Management procedures have been satisfactory and there has been no clinical evidence of nutritional deficiencies. Pancreatic tissues were collected from 99 chickens representing 10 farms,† in order to compare them with Laboratory chickens.

Materials and Methods

The data on lines and families in respect to (1) size of the original population, (2) mortality associated with gross lymphoid tumors, (3) mortality associated with other causes of death, and (4) survivors from which tissues were and were not taken, are given in Table I. As shown in the table, materials and data were obtained from 14 inbred lines of an F_1 cross (9×10) of 2 of these lines. All stock since the beginning of work at the Laboratory has been kept in complete confinement and no new breeding stock has been introduced since the first eggs were hatched.[3] Under "dead with gross lymphoid tumors" (D.W.L., Table I) is included one case of osteopetrosis, line 2, family 5, which will be treated statistically as lymphomatosis. Data on causes of death were taken from necropsy records compiled by the Laboratory veterinarians.

Of the 319 survivors, from which tissues were taken, 14 had lymphomatosis. The distribution of these birds and the number in each group are indicated in Table I by the numerals in parentheses.

"Other deaths" is a term applied to birds which died before the termination date and which at necropsy showed no gross evidence of lymphomatosis or osteopetrosis. Thus other deaths include a miscel-

* After this manuscript was completed, another report appeared on the subject: Lush, J. L., Lamoreux, W. F., and Hazel, L. N. The heritability of resistance to death in the fowl. *Poultry Sci.*, 1948, 27, 375–388. They state that "There is a genetic correlation of +.54 between resistance to the leukosis complex and resistance to death from other causes."

† Farm will be capitalized throughout the remainder of the article to signify this particular sample of chickens.

laneous assortment of diagnoses. From unpublished data prepared by Dr. Norman M. Nelson for the 1944 population, the sequence in the incidence from high to low is as follows: peritonitis, diagnosis undetermined, cannibalism, neoplasms other than lymphomatosis, reproductive disorders, traumatic injuries, coccidiosis, anomalies, hemorrhage, and hematomas. There were numerous additional causes of death each of which did not involve more than 1 per cent of the total mortality. In the organization of the data, therefore, lymphomatosis may be combined with another cause of death; whereas those listed under other deaths contain no cases showing gross lymphoid tumors. This introduces a bias against the analysis of other deaths, which cannot be adequately eliminated at the present time.

The family size varied from 1 to 26 (average 12), and the line size from 21 to 125 (average 75). In all, 1,123 birds were utilized for this analysis. Of these, 352 survived until termination date, a period which averaged 613 days. The range in age of the birds at termination was 541 to 764 days, and the average termination date for each line and the cross is shown in Table I.

The 99 Farm chickens (Table II) represented 10 farms within a 10-mile radius of Hamilton, Michigan.* Information concerning incidence of lymphomatosis in the farm populations from which birds were received was not obtained, nor are any data available on source of chicks, purity of breeds, or management practices. No gross lesions suggestive of lymphomatosis were observed during evisceration but

TABLE II

Number of Farm Birds from Which Tissues Were Taken

Age	Roaster					Fowl
Breed	Rhode Island Red		Barred Plymouth Rock		White Plymouth Rock	White Leghorn
Sex	M	F	M	F	M	F
Farm 1			6	4		
2						10
3			5		5	
4	10					
5					10	
6			10			
7					9	
8	10					
9	8	1	1			
10			2		8	
Total	28	1	24	4	32	10

* We are indebted to Mr. A. G. Lohman of the Hamilton Farm Bureau, Hamilton, Michigan, who segregated 10 birds from each of 10 farms and showed us many courtesies which facilitated the collection of tissues.

the liver was not seen in most cases, nor was there an opportunity to examine the kidneys or the usual complement of nerves. The average size of the spleen was larger than in the Laboratory chickens. It is obvious from the way in which the data fell (Table II) that age, sex, breed, and farm are confounded in varying degrees.

Single sections of the pancreas were made from the 319 Laboratory chickens and 2 sections, 1 to 3 mm. apart, were taken from each of the 99 Farm birds. Tissues from all chickens were fixed in Petrunkevitch no. 2,[5] sectioned at 5 μ, and stained with Galigher's hematoxylin and triosin.[6]

The procedure used to obtain the area of section and the size of lymphoid involvement was given in the preceding paper[1] and all figures are in square millimeters for the actual size of the object under consideration. These data were used to study number and size of individual lymphoid foci and the percentage of the section area involved. In Text-Figure 1 the amount of lymphoid tissue in the sections from selected cases is shown.

Observations

Relation of Lymphomatosis to Other Deaths in the Population

The bias present in the data, in that lymphomatosis-positive birds may include other causes of death but other deaths do not include cases of lymphomatosis, has necessitated consideration so that interpretation of these data will not lead to erroneous conclusions.

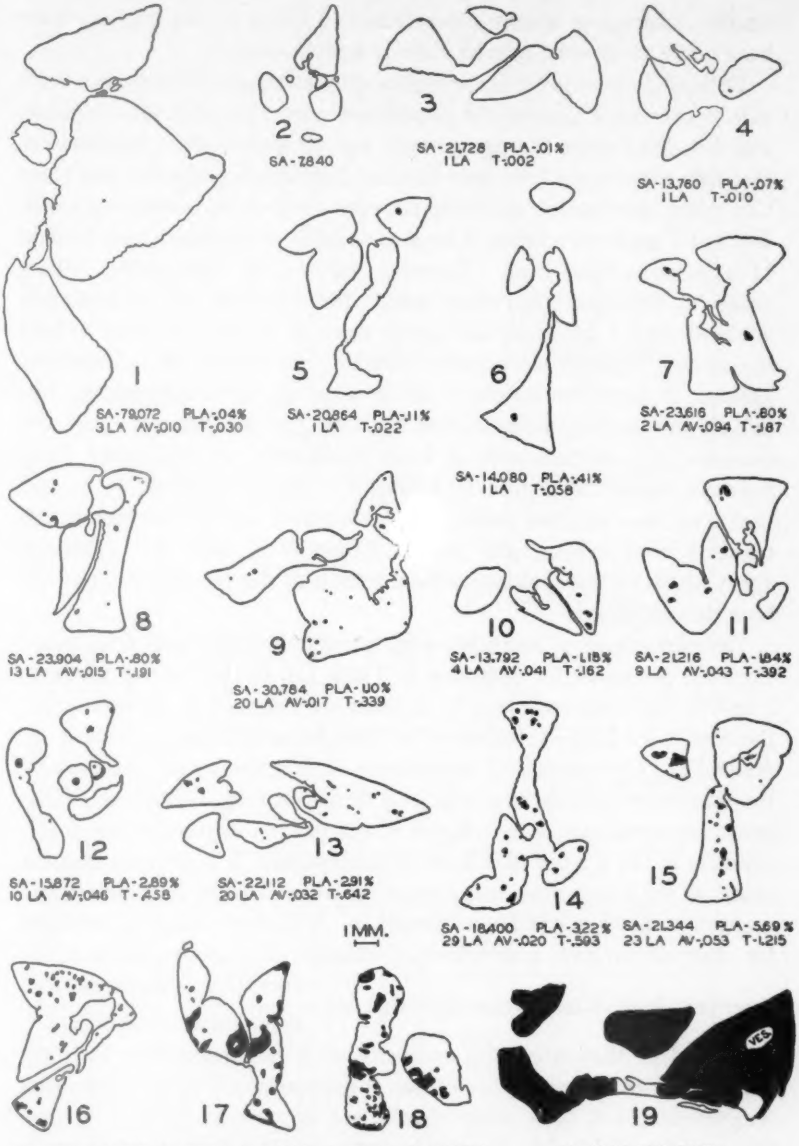
The following two methods were investigated to determine which gives the more exact measure of the percentages of lymphomatosis and of other deaths in families and lines:

Method I $\dfrac{\text{Lymphomatosis}}{\text{Population}} \times 100$ and $\dfrac{\text{Other deaths}}{\text{Population}} \times 100$

Method II

$$\frac{\text{Lymphomatosis}}{\begin{array}{c}\text{Population minus}\\ \text{other deaths}\end{array}} \times 100 \text{ and } \frac{\text{Other deaths}}{\text{Population minus lymphomatosis}} \times 100$$

In method I two errors are prominent: (1) Other deaths are treated as if the individuals involved survived to the termination date; whereas birds are dying without gross lymphomatous lesions at all ages. (2) When percentages for lymphomatosis are above 50 per cent, other deaths must automatically be less than 50 per cent; for example, line 12 had 77 per cent deaths with lymphomatosis and 23 per cent other



Text-Figure 1. The line drawings are of projected sections of pancreas from selected cases. The lymphoid areas are shown in black. The magnification is indicated by the millimeter scale. Abbreviations are as follows: SA = section area, PLA = percentage of lymphoid area, 3LA = 3 lymphoid areas, T = total lymphoid area. All areas are given in sq. mm.

deaths; whereas in reality other causes of death in line 12 may have been equal to or even greater than lymphomatosis.

Both of these objections are removed by method II in that each variable is computed against the population minus the alternate variable, and for this reason the percentages are somewhat more independent and each may range from zero to 100. Neither procedure is free from bias when correlations are made between the two variables concerned. Method I tends to produce a zero or negative correlation, and method II a positive correlation. However, method II theoretically allows points to fall almost anywhere within the space set by the ordinates while method I automatically limits them to a triangle equal to half the space. Method II raises a question concerning the influence of ageing. If there are no survivors in a group, one automatically obtains a perfect correlation in that both are 100 per cent. It raises the question, are chickens old, at least biologically, at 600 days? They have not passed the reproductive period of life so it can hardly be said that they have reached senility. We shall have no final answer to the question until it is possible to test longevity in birds free from the agent which causes lymphomatosis, and up to the present this has not been accomplished.

The percentage of mortality with lymphoid tumors and from other causes is presented for each line in Table III as obtained by methods I and II. The lines and the 9×10 cross are arranged in sequence from the lowest to highest incidence of lymphomatosis as calculated by method II. Comparison of percentages of lymphomatosis obtained by the two methods indicates relatively little shifting in position of the lines; the coefficient of correlation was 0.760 (13 degrees of freedom), which is at the 0.1 per cent level of significance. It is obvious that for practical purposes, when lymphomatosis alone is being considered, one method is about as reliable as the other. A similar method of analysis for other deaths gave a correlation coefficient of 0.646, which is at the 1 per cent level. Ratios were calculated as: $\frac{\text{percentage, lymphomatosis}}{\text{percentage, other deaths}}$. It is evident that when the incidence of lymphomatosis is low, the number of deaths from other causes is proportionally larger and when lymphomatosis is high, other deaths are proportionally less. A certain amount of this shift in ratio is due to the fact that lymphomatosis deaths include also some other causes of death and also because the original population and survivors are common factors to the two variables which are being considered.

It is noteworthy that ratios for the 9×10 cross in the group with

TABLE III

Ratios for Each Line of Deaths with Lymphoid Tumors to Other Deaths by Methods I and II at Termination (613 Days)

Lines arranged in sequence of increasing percentage of lymphomatosis by method II		Method I: Percentage of lymphomatosis	Method I: Percentage of other deaths	Method I: $\dfrac{\dfrac{\text{Lymphomatosis}}{\text{Population}} \times 100}{\dfrac{\text{Other deaths}}{\text{Population}} \times 100}$	Method II: Percentage of lymphomatosis	Method II: Percentage of other deaths	Method II: $\dfrac{\dfrac{\text{Lymphomatosis}}{\text{Population}-\text{other deaths}} \times 100}{\dfrac{\text{Other deaths}}{\text{Population}-\text{lymphomatosis}} \times 100}$
Lines having less than 50 per cent of lymphomatosis (method II)	6	8	13	.62	9	14	.64
	7	24	27	.89	33	35	.94
	10	24	32	.75	36	43	.84
	1	20	47	.43	37	59	.63
	9 × 10	34	15	2.27	40	23	1.74
	13	25	43	.58	44	57	.77
	5	29	38	.76	46	60	.87
				Average .90			Average .92
Lines having more than 50 per cent of lymphomatosis (method II)	15	50	22	2.27	64	43	1.49
	4	46	30	1.53	65	54	1.20
	3	56	17	3.29	67	35	1.72
	11	42	45	.93	77	78	.99
	14	49	37	1.32	78	73	1.07
	9	47	41	1.15	80	77	1.04
	2	54	44	1.23	96	95	1.01
	12	77	23	3.35	100	100	1.00
				Average 1.88			Average 1.19

less than 50 per cent lymphomatosis and line 11 (which was crossed with line 2, 2 years previously) show the greatest divergences from other lines in the respective groups. Interpretation of these results is not possible with the limited data available.

Age at death was investigated in order to disclose possible biases due to taking the sample at the termination date. The average age at death on a line basis for lymphomatosis and for other causes of death is shown in Table IV. Considerable variability is evident among lines.

TABLE IV

Mean Age at Death for Lines in Respect to Lymphomatosis, Other Deaths, and Total Mortality, When the Holding Period Average is 613 Days

Lines arranged in sequence of increasing percentage of lymphomatosis by method II		Lymphomatosis	Other deaths	Total mortality
Lines having less than 50 per cent of lymphomatosis (method II)	6	426	424	425
	7	225	339	288
	10	389	257	314
	1	313	293	299
	9×10	379	264	343
	13	472	376	410
	5	225	231	228
Average		342	316	328
Lines having more than 50 per cent of lymphomatosis (method II)	15	246	293	261
	4	339	344	341
	3	338	333	337
	11	331	291	310
	14	282	272	277
	9	245	248	248
	2	231	254	241
	12	326	375	338
Average		293	290	292
Average for all		306	300	303

Under the column, total mortality, the more resistant lines lived somewhat longer on the average than the more susceptible lines. Also when the averages under the two columns, lymphomatosis and other deaths, are compared, it would appear that, in the resistant half of the population, the survival time was longer when associated with lymphoid tumors than when associated with other causes of death. The same comparison made in the susceptible half of the population showed very little difference. For the whole population the average age at death for lymphomatosis and other causes is nearly the same, 306 and 300 days respectively.

Variance analysis for age at death in respect to lines and cause (Table V) shows a significant difference between lines but not between causes of death. The latter might have been expected from the averages for the whole population shown in Table IV. The inter-

action of lines and causes of death was not calculated because of the great difference in subclass numbers. Data in Table IV indicate considerable fluctuation within both resistant and susceptible lines, but

TABLE V

Analysis of Variance for Age When Dead with Lymphoid Tumors and for Other Deaths

Source of variance	Degrees of freedom	Mean square
Total	770	
Lines	14	123,875*
Dead with lymphoid tumors *vs.* other deaths	1	6,091
Within lines and within causes of death	755	19,537

* 1 per cent level of significance.

there is a tendency for age at death from lymphomatosis to be higher than from other causes of death in more resistant lines and equal to other causes of death in the more susceptible group.

As already mentioned, method II biases correlations in a positive direction when lymphomatosis and other causes of death are compared. This bias can be lessened if the data are broken down into small age groups. The percentages of death from these two causes were calculated for 100-day age periods (Table VI). A new population was deter-

TABLE VI

Using Method II—Percentage of Dead with Lymphoid Tumors and Other Deaths for 100-Day Age Intervals

Age in days	Population at beginning of each age period	Population minus other deaths	Percentage of dead with lymphoid tumors	Population minus dead with lymphoid tumors	Percentage of other deaths
30–100*	1123	1090	2.2	1099	3.0
101–200	1066	984	10.2	966	8.5
201–300	884	824	12.6	780	7.7
301–400	720	645	12.4	640	11.7
401–500	565	499	12.0	505	13.1
501-to termination (613)†	439	408	13.7	383	8.1
Calculated 501–600†	439	412	12.1	389	6.9

* Chicks from hatching through 29 days were not included.

† Average age at termination was 613 days. The calculated value is the observed figure × 100/113.

mined for the beginning of each age period. The percentage of death up to 100 days is low from both causes. Had chick mortality been included, other deaths would have been somewhat higher. After 100 days, the percentage of lymphomatosis remains remarkably constant. A χ^2 test indicated no significant differences in percentage of mortality from lymphomatosis for the 100 to 600 day age group, but there were significant differences in percentage of mortality from other causes. However, it can hardly be asserted that mere ageing of the birds is a

major contributing factor to other deaths. If it were, then the last age period would probably have had a much higher value than it did unless there is a hump in the mortality curve for chickens. Little data on this are available. Data presented by Gardner and Hurst[7] show only a smooth S-shaped curve.

In a further effort to determine whether there is a relationship between lymphomatosis and other causes of death, the number of deaths per line from 100 to 500 days was treated as four age groups of 100 days each. This procedure does not require the use of either method I or II. The 0 to 100 day period was omitted because autopsy records were not kept prior to 30 days; the 500 to 600 day period was excluded because some birds were killed before they were 600 days old. Correlations were based on the numbers dead per line per age group and were weighted for the size of the line populations available at the beginning of each period. A significant correlation coefficient of 0.276, 58 degrees of freedom, was obtained for the resulting 60 groups (Table VII). The r value of 0.600, 13 degrees of freedom, which was obtained

TABLE VII

Correlations of Mortality from Lymphomatosis and from Other Deaths by 100-Day Periods, Covering the Age Span of 100 Through 500 Days, Based on Covariance Analysis

Source of variation	Degrees of freedom	Coefficient of correlation
Total	58	.276*
Lines	13	.600*
Periods	3	.000
Remainder	41	.056

* 5 per cent level of significance.

for lines, was significant at the 5 per cent level; correlations between periods and between periods within lines were approximately zero.

Therefore, it appears that the information obtained from analyses made thus far clearly demonstrates that the segregation of the population into lines resistant and susceptible to lymphomatosis has resulted in a parallel segregation of incidence due to other causes of death. Whether there is a direct causal relationship between the agent producing lymphoid tumors and other manifestations of morbidity, or an indirect relationship stemming from the bird's genetic constitution cannot be settled with the present data. The answer may come when the agent or agents which are responsible for the naturally occurring disease have been identified and when less arbitrary and subjective methods have been worked out for the diagnosis of the disease. The agent or agents will probably be identified when lymphomatosis is approached as a systemic rather than a neoplastic disease. Which is

the more direct cause of death, the lymphoid tumor itself, or the agent causing the tumor? It is entirely possible that chickens classified at necropsy as dying from an undetermined cause of death may have died in some instances from the agent of lymphomatosis before any lymphoid tumors reached visible size. Probably not all causes of other deaths are associated with lymphomatosis to an equal degree. Moore[8] concluded that one cause of mortality, ruptured yolk, occurred in association with fowl paralysis more frequently than it would by chance alone.

A problem which is antecedent to the use of these data for lymphoid tissue studies is the uniformity of performance of families within lines and the 9 $\times$ 10 cross. Results of χ^2 tests on each line are shown in Table VIII. Lymphomatosis mortality for most families within lines and the 9 $\times$ 10 cross is not significantly different, but for lines 3 and 15 it is beyond the 1 per cent level of significance. Line 15 is the most variable and the basis of this extreme variability is brought out in Table I in which lymphomatosis in families ranges from 7 to 85 per cent (method I) or 10 to 100 per cent (method II). The variability among families for other causes of death is highly significant only for the 9 $\times$ 10 cross. When the data are analyzed for total mortality (Table VIII) the families of lines 15 and the 9 $\times$ 10 cross show highly significant variations but not for families within line 3.

Variables Involved in the Study of Lymphoid Tissue

The histologic quantitative data deal with four variables: (1) the size of the section, (2) the percentage of lymphoid tissue per section, (3) the number of lymphoid areas per section, and (4) the size of individual lymphoid areas. The selected sections, shown in Figures 1 to 19 of Text-Figure 1, show the ranges for these variables. The largest cross section encountered is shown in Figure 1, and the smallest in Figure 2. There is approximately a 10-fold difference. Figures 2 to 19 are arranged in order of increasing percentage of lymphoid tissue, which varies from zero to 88.37 per cent. The number of lymphoid areas in the cross section varied from none (Fig. 2) to 36 (Fig. 18). The smallest lymphoid area was 0.00037 sq. mm. and it contained about 9 cells. The largest is shown in Figure 19, in the portion with the label VES, and it has an area of 16.26 sq. mm. Between the smallest and the largest there is approximately a 44,000-fold difference in size.

It is quite evident from an examination of the figures that none of the variables are automatically linked to one another. For example,

number and amount would seem to be logically related, and it is evident from the illustrations that, in general, they are correlated, yet Figures 7 and 8 illustrate a wide divergence. In Figure 7 there are but two lymphoid areas and in Figure 8 there are thirteen, yet they both

TABLE VIII

Results of χ^2 Test for Uniformity of Performance of Families Within Lines in Regard to Mortality from Lymphomatosis and to Other Deaths

		Method II				Method III	
		Lymphomatosis		Other deaths		Total mortality	
		Population—other deaths		Population—lymphomatosis		Population	
Line	Degrees of freedom	χ^2	P	χ^2	P	χ^2	P
1	4	3.598	.48	1.230	.87	2.496	.65
2	6	12.163	.06	4.463	.62	13.880	.03
Y.C.						8.199	.23
3	8	25.163	.002	6.026	.65	20.159	.01
Y.C.		16.096	.005			13.083	.11
4	8	13.400	.09	11.062	.20	15.546	.05
Y.C.						10.592	.23
5	5, 4, 6	3.412	.66	5.432	.25	4.802	.57
6	4	11.217	.03	3.878	.43	8.570	.08
Y.C.		6.280	.18				
7	9	16.273	.06	2.256	.99	6.229	.72
9	3	9.576	.02	7.452	.06	9.245	.03
Y.C.		5.777	.13	4.431	.22	5.679	.14
10	5	3.025	.56	6.706	.25	4.184	.52
11	6	8.582	.20	7.647	.27	9.399	.16
12	2	0.000	1.00	0.000	1.00	0.000	1.00
13	5	7.640	.18	6.015	.31	9.879	.08
14	5, 4, 5	7.234	.20	8.978	.06	9.891	.08
15	5	36.543	.001	7.561	.19	29.019	.001
Y.C.		29.413	.001			22.956	.001
9×10	5	14.223	.02	20.683	.001	19.013	.003
Y.C.		10.269	.07	15.663	.01	15.356	.01
Between lines	14	160.066	.001	130.678	.001	180.127	.001

df = For lines 5 and 14, the df are enumerated in order of each analysis; for all other lines the same degrees of freedom were used throughout.

Y.C. = Yates correction for small numbers.

occupy 0.8 per cent of the section. It is obvious that an increase in percentage may entail a reduction in number of lymphoid areas, since small areas such as shown in Figures 13 and 14, which are close together, undoubtedly fuse as they grow larger, and thereby the number

is reduced. Thus number and percentage of lymphoid tissue may be antagonistic, as well as positively correlated. The net effect is the algebraic sum of these two sets of interacting forces. A quantitative study of this subject is presented later.

The distribution of the lymphoid foci throughout the section is entirely a random one as may be seen in Figures 1 to 19 except in so far as such foci are often associated with blood vessels.

Percentage of Lymphoid Tissue and Effect of Lymphoid Tissue on Section Areas

$$\frac{\text{Total lymphoid area}}{\text{Section area}} \times 100$$ gave the percentage of lymphoid tissue for each section studied. Table IX and Text-Figure 2 show the num-

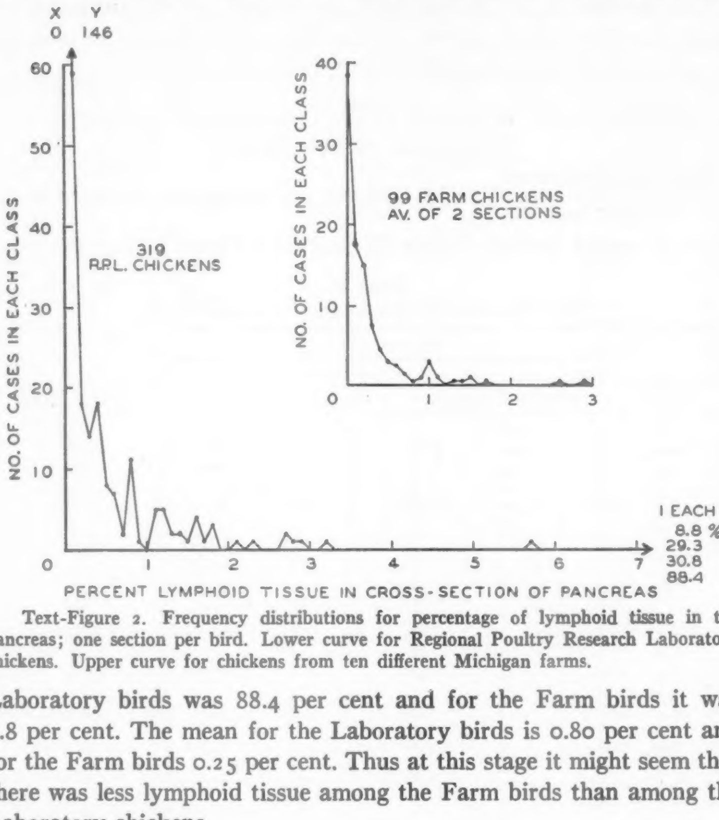
TABLE IX

Class Frequencies for Percentage of Lymphoid Tissue

Percentage classes	Number of cases			Value based on sum of A and B sections
	Laboratory chickens	Farm chickens		
		A section	B section	
.0	126	28	32	15
Trace	20	9	8	17
.1	59	18	17	24
.2	18	15	15	19
.3	14	9	6	5
.4	18	6	3	3
.5	8		6	2
.6	7	2	3	3
.7	2	1	2	2
.8	11	1		3
.9	1		2	
1.0		4	2	1
1.1	5	2		
1.2	5			1
1.3	2	1		2
1.4	2		1	1
1.5	1	1	1	
1.6	4			
1.7	1	1		
1.8	3			
2.1	1			
2.3	1			
2.6			1	
2.7	2			
2.8	1			1
2.9	1	1		
3.2	1			
5.7	1			
8.8	1			
29.3	1			
30.8	1			
88.4	1			
Total	319	99	99	99

ber within each class range. No lymphoid tissue occurred in 126 sections and in 20 more there was a trace (0.001 to 0.050 per cent). Thus

the curve originates at 146 on the Y axis. The incidence in each succeeding class dropped rapidly and soon extended to an extremely long tail, approaching the X axis. The Farm birds showed a similar J-shaped curve but without as long a tail. The maximum value obtained in the



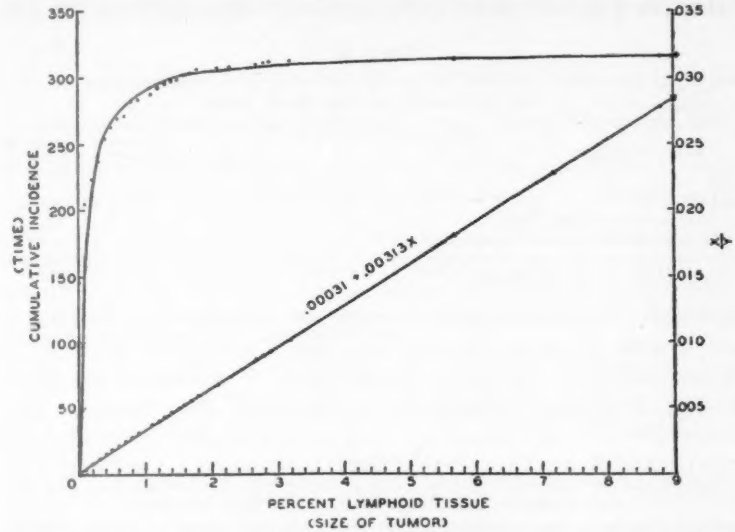
Text-Figure 2. Frequency distributions for percentage of lymphoid tissue in the pancreas; one section per bird. Lower curve for Regional Poultry Research Laboratory chickens. Upper curve for chickens from ten different Michigan farms.

Laboratory birds was 88.4 per cent and for the Farm birds it was 2.8 per cent. The mean for the Laboratory birds is 0.80 per cent and for the Farm birds 0.25 per cent. Thus at this stage it might seem that there was less lymphoid tissue among the Farm birds than among the Laboratory chickens.

The J-shaped curves do not fit Poisson distributions for the respective means. The curve for Laboratory chickens extended too far along the axes and in the region of greatest curvature lies well below the theoretical curve. The Farm birds showed a distribution which more closely approached a Poisson series.

Another method of analysis was attempted in which the cumulative Y values were plotted against the X values (Text-Figure 3). Then the points for $\frac{X}{\text{cum.}Y}$, X were determined and these were found to fall almost exactly on a straight line. By the method of least squares the

regression line for these points was calculated and from values of selected points a hyperbola was constructed. The cumulative curve and the calculated hyperbola closely approximated each other, which may be interesting but did not lend itself to comparisons of different groups



Text-Figure 3. The points along the straight line were obtained by plotting the class value x (abscissa) against $\frac{x}{y}$ (ordinate to the right), y is a cumulative frequency value. By the method of least squares a regression line was calculated having the slope given for $y+bx$. Cumulative frequency values (ordinate to the left) are plotted along the curved line as points against x. The solid curved line is a calculated hyperbola derived from the slope of the regression line. The ordinates (size of tumor and time) apply to a theoretical discussion of the hyperbolic curve. The arrows indicate that the curves should be extended to 88.4 per cent. Values above 9 per cent were found to fall close to the theoretical solid lines.

of birds. The regression line had some value for comparative purposes but required large numbers to establish accurately the slope of the line. Variance analysis of normalized data was made of Laboratory birds to determine if there were significant differences between lines and between families within lines. That significant differences probably existed between lines was suggested by the variations in the curves for percentage of lymphoid tissue. Line 13 and the cross, 9 × 10 (Text-Fig. 4), showed the extremes for the percentage of lymphoid tissue. The results of the analysis are given in Table X and show that there are highly significant differences between families within a line as well as between lines. The latter was expected and the former might have been anticipated from the analysis given earlier

in this paper (Table VIII) in which line 3 and especially line 15 had a significant variability among families in regard to their development of lymphoid tumors.

The next approach to the problem was to determine whether the amount of lymphoid tissue in the survivors was related to the performance of families and lines in respect to mortality from lymphomatosis, to other deaths, and to total mortality. The results of the analyses are summarized in Table XI. Higher levels of correlation are found when lymphoid tissue is compared with mortality from lymphomatosis than when it is compared with other deaths. The only significant figure in the column under other deaths is barely at the 5 per cent level.

TABLE X

Variance Analysis Based on Coded Values for Percentage of Lymphoid Tissue for Lines and for Families of Laboratory Birds

Source of variation	Degrees of freedom	Mean square
Total	318	
Between lines	14	4.774*
Between families within lines	60	1.130*
Between birds within families within lines	244	0.567

* 1 per cent level of significance.

TABLE XI

Association of Percentage of Lymphoid Tissue in Survivors with Percentage of Mortality from Lymphomatosis, Other Deaths, and Total Mortality

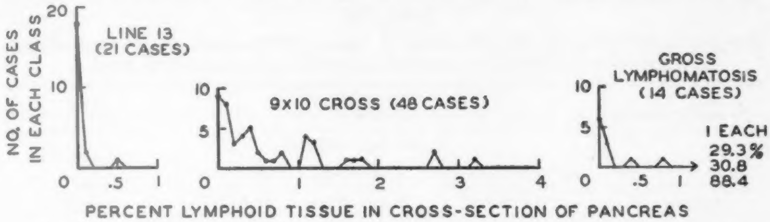
	Correlations between (1) and (2)			
	(1) Percentage of deaths with lymphoid tumors (method II) and (2) coded values of percentage of lymphoid tissue in survivors		(1) Percentage of other deaths (method II) and (2) coded values of percentage of lymphoid tissue in survivors	
	Families df=73	Lines df=13	Families df=73	Lines df=13
r Based only on families from which tissues were taken	.2661*	.6067*	.1979	.4696
r Based on all families		.6625†		.5157*

* 5 per cent level of significance.
† 1 per cent level of significance.

Before analyses were made it was anticipated that correlation for families would be greater than for lines, since the family is genetically more homogeneous. The lower values actually observed for families may have been due to uncompensated errors resulting from percentages based on small numbers or due to restriction of the sample. The important point is that a small sample of tissue, taken in every case

from a grossly negative organ and from 28 per cent of the original population, reflects the behavior of the population in relation to development of grossly visible lymphoid tumors.

The Farm birds were studied in a similar way in order to obtain some information on how breed, sex, age, and the average farm environ-



Text-Figure 4. Frequency distribution of percentage of lymphoid tissue for three selected groups of Laboratory chickens. Although 9 x 10 cross was grouped with the resistant chickens, it has since been shown to have significantly more lymphoid tissue than even the average for the susceptible lines.[15]

ment might modify the lymphoid reactions. The distribution among breed, age, sex, and farm is given in Table II, the incidence of various percentages of lymphoid tissue in Table IX, and the distribution curve in Text-Figure 2. Since two sections were cut from different regions of the same block they have been designated A and B. This material aids us in testing an assumption which up to the present has been tacitly accepted, namely, that the lymphoid tissue was uniformly distributed through the organ and that one section was therefore representative. A correlation of percentage of lymphoid tissue for the A and B sections gave an r value of 0.8613 and 97 degrees of freedom. This exceedingly high correlation justified the use of only a single section as was done with the Laboratory chickens. Average of the A and B sections was used for coding. Analysis of variance with the use of coded values for percentage of lymphoid tissue is given in Table XII, section A, for farms, breeds, sex, and age. Highly significant values were obtained for farms, breed, and age, but not for sex. However, the first three variables are so badly confounded as seen in Table II that these results have little meaning. The chickens on farm 2 had less lymphoid tissue than those on any of the other farms, but was it due to less lymphomatosis or to the White Leghorn breed which was obtained only from that farm, or to the fact that they were older birds, a condition which also was limited to that farm?

An attempt was made to break down a portion of the analysis (Table XII, section B) by limiting it to males and to breeds of roaster age. On that basis there was no significant difference between breeds or farms.

However, farm differences closely approached the 5 per cent level; breed differences did not.

The next step in the analysis deals with the question: Do the Laboratory birds, perhaps with a higher degree of inbreeding and a higher degree of freedom from other diseases, have more or less lymphoid

TABLE XII

Analysis of Variance with the Use of Coded Values of Percentage of Lymphoid Tissue as Found in Chickens from 10 Farms, 4 Breeds, 2 Ages, and Both Sexes

Source of variation	Degrees of freedom	Mean square
	A: All data utilized	
Total	98	
Farm	9	2.50*
Error	88	.818
Total	98	
Breed	3	4.12*
Error	95	.864
Total	98	
Age	1	9.15*
Error	97	.879
Total	98	
Sex	1	2.67
Error	97	.946
	B: Analysis limited to farms having males of roaster age	
Total	83	
Farm	8	1.66
Error	75	.82
Total	83	
Breed	2	1.26
Error	81	.89

* 1 per cent level of significance.

tissue than the average Farm birds? New coded values had to be set up for the combined population of Laboratory and Farm birds. For this study it was decided to use only the A section of the latter in order to make the samples of comparable size. Some of the data relative to the study are set up in Table XIII. There were 200 survivors, from which tissues were taken, involved in the lines having less than 50 per cent lymphomatosis and 119 in those having more than 50 per cent. The means for the various groups are given in Table XIII.

The Farm birds had a mean of 1.09. Analysis of variance for all Laboratory *vs.* all Farm birds showed no significant difference between the two groups of chickens (Table XIV), but when the Laboratory birds were subdivided into those lines having less than 50 per cent lymphomatosis and those having more, a variance analysis gave highly significant differences at well beyond the 1 per cent level, and from the

means it is evident they fall in the following decreasing sequence: Laboratory resistant birds, Farm birds, and Laboratory susceptible birds.

Thus, it may be concluded that as far as lymphoid tissues are concerned, the average picture for the Laboratory birds, after 7 years of

TABLE XIII

Percentage of Lymphomatosis, Percentage of Other Deaths, and Mean Coded Values of Lymphoid Tissue for Lines of Laboratory Birds; Also Mean Coded Values of Lymphoid Tissue for Farms

Lines arranged in sequence of increasing percentage of lymphomatosis by method II	Percentage of lymphomatosis by method II	Percentage of other deaths by method II	Mean coded values for lymphoid tissues in pancreas	Mean coded values for lymphoid tissue for the group
	Less than 50 per cent of lymphomatosis			
6	9	14	.52	
7	33	35	.50	
10	36	43	.89	
1	37	59	1.03	
9 × 10	40	23	1.58	
13	44	57	.20	
5	46	60	1.57	
				Line basis .90
				Bird basis .87
	More than 50 per cent of lymphomatosis			
15	64	43	1.23	
4	65	54	.75	
3	67	35	1.46	
11	77	78	1.42	
14	78	73	1.32	
9	80	77	1.87	
2	96	95	1.40	
12	100	100	2.19	
				Line basis 1.46
				Bird basis 1.24
				All lines 1.20
				All birds 1.00
Farm numbers				
1			1.33	
2			.40	
3			1.52	
4			1.54	
5			.62	
6			.80	
7			1.58	
8			1.15	
9			1.05	
10			.92	
				Farms and birds 1.09
				Bird basis
				Lab. and Farm 1.02

inbreeding, is still the same as that for the Farm flocks which were examined. Selection, however, has pushed the lymphoid picture in opposite directions just as it has the development of grossly visible lymphoid tumors.

The hypothesis has already been developed that the amount of lymphoid tissue in well birds is a reliable measure of the incidence of lymphomatosis for their genetic group, and it likewise automatically becomes a measure of resistance and susceptibility if a constant dose factor of the agent is assumed. A criterion is needed to distinguish

TABLE XIV

Analysis of Variance of Coded Percentages of Lymphoid Tissue for Laboratory and Farm Chickens

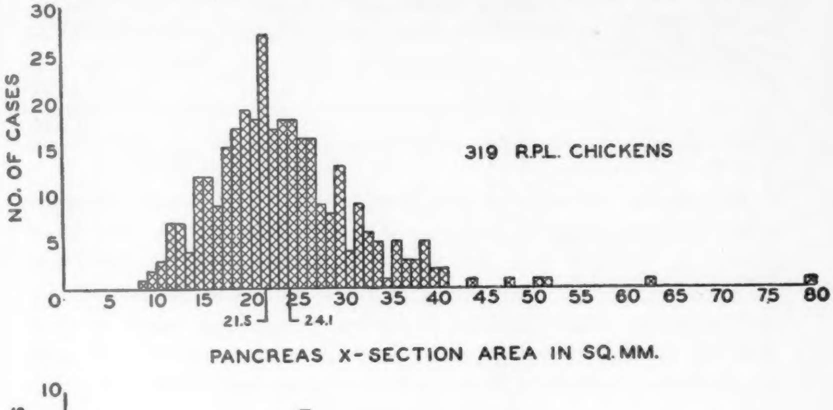
Source of variation	Degrees of freedom	Mean square
Comparison of lymphoid tissue in (1) all Laboratory chickens and in (2) Farm chickens		
Total	417	
Between 2 groups	1	.5215
Error	416	.8920
Comparison of lymphoid tissues in (1) birds of Laboratory lines having less than 50 per cent of lymphomatosis, (2) same having more than 50 per cent, and (3) Farm chickens		
Total	417	
Between 3 groups	2	5.3431*
Error	415	.8697

* 1 per cent level of significance.

between a tissue response due to a low dose factor and that due to resistance. At present they appear to be the same and perhaps the low lymphoid response in line 13 associated with moderately high lymphomatosis mortality may be due to a low dose factor.

It is well known that lymphomatosis often causes enlargement of the liver. A similar reaction has not usually been reported for the pancreas but if it does enlarge the organ the amount of enlargement is rarely great enough to be recognized at necropsy. The distribution of pancreatic cross-sectional area is given in Text-Figure 5 for Farm and Laboratory chickens. The range is slightly greater in the latter and there is a slight skewness in both curves with a more pronounced tail to the right in the Laboratory birds. Means for lines, Farms, and other groups are shown in Table XV. There is considerable variability in means for Laboratory lines and Farms. The pancreatic cross-sectional area is greater in resistant birds than in susceptible ones. The area for Farm chickens is greater than for Laboratory birds. In both, the pancreas cross section is larger for those which showed

lymphoid areas than for those which were negative. A correlation made on Laboratory birds between section size and amount of lymphoid tissue, excluding sections which had no lymphoid tissue, gave an r value of 0.1307 (191 degrees of freedom), which lies at the 5 per cent level. But because lines were not equally represented, there may have



Text-Figure 5. Histograms of pancreas cross-sectional areas for Laboratory and Farm chickens. Lines below abscissae point to mean values. Means to the left are for sections without lymphoid areas and those to the right are for those with lymphoid areas.

been a bias and the analysis of variance given in Table XVI was made. Differences between lines are highly significant for section size and likewise there is a highly significant effect of lymphoid tissue on section size. Interactions were pooled with the error term. A somewhat similar analysis for Farm birds was made. A correlation of size of A sections (including only those with lymphoid tissue) with the amount of lymphoid tissue gave an r value of 0.269 (69 degrees of freedom) and the same for B sections gave an r value of 0.229 (66 degrees of freedom), both of which are close to the 5 per cent level. An analysis of variance (Table XVII) showed significance between the 1 and 5 per cent levels when section size in relation to presence or absence of

lymphoid tissue was considered. It was decided to group the other variables of sex, age, farm, and breed in the "error" term because of confounding.

TABLE XV

Mean Size of Pancreatic Areas for Various Groups

Laboratory lines*	Mean in sq. mm.	Farms† nos.	Mean in sq. mm.
Less than 50 per cent of lymphomatosis			
6	19.93	1	22
7	18.79	2	17
10	27.77	3	32
1	23.88	4	27
9 × 10	24.85	5	22
13	23.64	6	27
5	26.04	7	28
		8	25
Av. on bird basis	23.60	9	26
		10	25
More than 50 per cent of lymphomatosis			
15	20.87		
4	31.01		
3	18.55		
11	24.37		
14	20.89		
9	24.33		
2	18.77		
12	16.00		
Av. on bird basis	21.80		
Average for all Laboratory birds			23.1 sq. mm.
Average for Laboratory birds with no lymphoid tissue in section			21.5 sq. mm.
Average for Laboratory birds with lymphoid tissue in section			24.1 sq. mm.
Average for all Farm birds			25.2 sq. mm.
Average for Farm birds with no lymphoid tissue in section			22.5 sq. mm.
Average for Farm birds with lymphoid tissue in section			26.2 sq. mm.

* Lines arranged in sequence from lowest to highest incidence of lymphomatosis as determined by method II.

† All data on Farm birds from "A" section.

The data, no matter how they were handled, seemed to indicate an increase in cross-sectional area associated with lymphoid tissue in spite of the fact that the more resistant lines had the larger mean section area. Such a result may indicate merely that the more tissue

TABLE XVI

*Analysis of Variance of Section Size in Relation to the Amount of Lymphoid Tissue and Lines for Laboratory Birds**

Source of variation	Degrees of freedom	Mean square
Total	318	
Sections with lymphoid tissue vs. sections without lymphoid tissue	1	558.43†
Section size for lines	13	394.11†
Error	304	51.31

* Neoplastic case in line 7 (Fig. 19 of Text-Fig. 1) omitted. It contains more lymphoid tissue than all other sections combined.

† 1 per cent level of significance.

one employs the more lymphoid tissue one will find, and this is further evidence for a uniform distribution of lymphoid tissue, at least up to the stage where it becomes wildly proliferative. It could also mean that

TABLE XVII

Analysis of Variance of Section Size in Relation to Amount of Lymphoid Tissue for Farm Birds

Source of variation	Degrees of freedom	Mean square
Total	98	
Sections with lymphoid tissue vs. sections without lymphoid tissue	1	275*
Error	97	51.5

* 5 per cent level of significance.

the more abundant lymphoid tissue is responsible for enlargement of the organ. At the present there is no way to make an analysis which will separate the variables of section size and lymphoid area sufficiently well to determine which is antecedent. At least, there is no obvious reason why accumulations of lymphoid tissue should not enlarge the pancreas in the same way it is known to enlarge the liver. Gross weights of internal organs of chickens are being collected in the hope that more exact information will be gained.

Size of Individual Lymphoid Areas

It was anticipated before the measurements were made that the lymphoid areas would fall into a distribution curve of normal type, but as seen in Table XVIII and Text-Figure 6, they produced a more exaggerated J-shaped curve than did the distribution of percentage of lymphoid tissue. In the 319 Laboratory chickens, 1,073 lymphoid areas were measured; in section A of the Farm birds, 348 areas; and in the B section, 312 areas. The curve shown in Text-Figure 6 for the Farm birds is an average of the A and B sections. The mean size of the lymphoid areas for the Laboratory birds is 0.032 sq. mm. and for the Farm birds it is 0.020 sq. mm. The average size found in the Laboratory chickens is 1.5 times larger than that obtained in the Farm chickens. As shown in Table XIX, the means for the lines show a 5-fold variation from smallest to largest. In the Laboratory flock the resistant lines have an average for size of lymphoid area about half as large as that for susceptible lines. When lines were divided into two groups at the 50 per cent level of lymphomatosis, the means were 0.024 and 0.046 sq. mm.

In order to determine whether mean differences were significant, the data for size of individual lymphoid areas were normalized and the values obtained were then used in making the analyses presented in

Table XX. The size of lymphoid areas in Farm chickens is significantly less than for Laboratory resistant *vs.* susceptible groups. It was surprising that there was no significant difference between lines, in view of the wide differences in line means. To test the reality of this a correlation was made between mean normalized values for the lines and the

TABLE XVIII

Class Frequencies for Size of Individual Lymphoid Areas

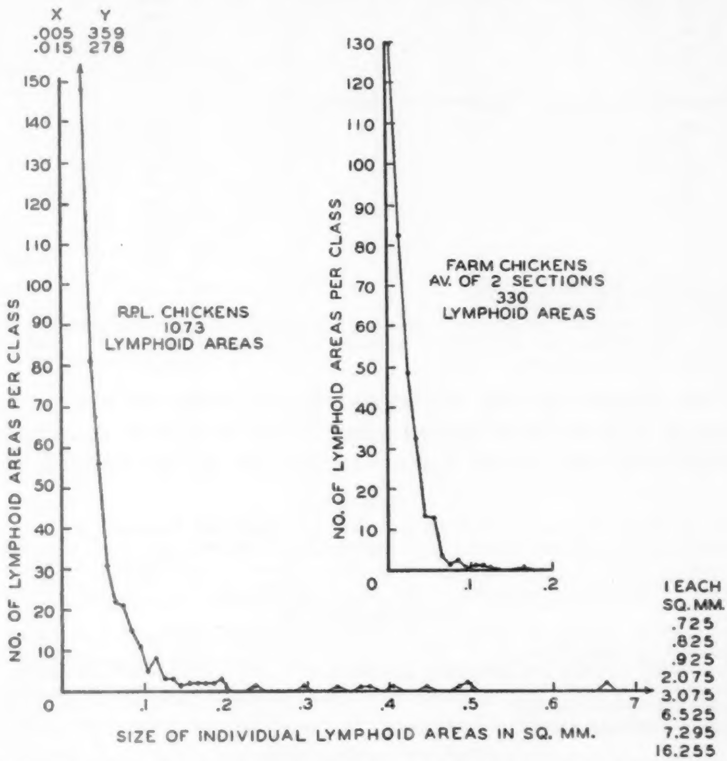
Size of lymphoid areas in sq. mm.	Number of lymphoid areas		
	Laboratory chickens	Farm chickens	
		A section	B section
.005	359	140	119
.015	278	76	89
.025	148	51	46
.035	81	41	24
.045	56	15	11
.055	31	12	14
.065	22	3	4
.075	21	2	1
.085	15	4	1
.095	11		1
.105	5	2	
.115	8	1	1
.125	3		1
.135	3		
.145	1		
.155	2		
.165	2	1	
.175	2		
.185	2		
.195	3		
.235	1		
.295	1		
.335	1		
.365	1		
.375	1		
.405	1		
.445	1		
.485	1		
.495	2		
.665	2		
.725	1		
.825	1		
.925	1		
2.075	1		
3.195	1		
6.525	1		
7.295	1		
16.255	1		
Total	1073	348	312

percentage of lymphomatosis. The r value was 0.0438 (13 degrees of freedom), which is not significant.

Thus the results on size of individual lymphoid areas agree with those on percentage of lymphoid tissue to the extent that resistant lines have values significantly lower than the susceptible lines. However, when Laboratory birds as a group are compared with Farm birds

the results are not in agreement in that, on the one hand, the mean sizes of individual lymphoid areas are significantly different, while on the other hand percentages of lymphoid tissue do not show a significant difference.

There still remains for consideration the problem of size of indi-



Text-Figure 6. Frequency distribution curves for size of individual lymphoid areas for Laboratory and Farm chickens.

vidual lymphoid areas as influenced by the number of such areas in the section. This is taken up as part of the next section.

Number of Lymphoid Areas

The curve for number of lymphoid areas in sections follows a J-shaped distribution as did the percentage of lymphoid tissue and the size of individual lymphoid areas. The data for Laboratory and Farm chickens are presented in Table XXI and Text-Figure 7. There is a slight difference in the shape of the two curves in that 39 per cent

TABLE XIX

Mean Size of Individual Lymphoid Areas for Various Groups

Lines	Mean in sq. mm.	
Less than 50 per cent of lymphomatosis (method II)		
6	.021	
7	.023*	
10	.015	
1	.030	
9 × 10	.020	
13	.032	
5	.025	.024
More than 50 per cent of lymphomatosis (method II)		
15	.079	
4	.063	
3	.034	
11	.021	
14	.040	
9	.029	
2	.028	
12	.023	.046
Average for all Laboratory birds		.032*
Average for all Farm birds		.020

* If the neoplastic case is included (Fig. 19 of Text-Fig. 1), it is .831 for line 7 and .065 for all Laboratory chickens.

of the Laboratory birds showed no lymphoid tissue and only 30 per cent of the Farm birds showed none. In the Farm birds the number of lymphoid areas for the A and B sections are highly correlated. The

TABLE XX

Variance Analysis of Normalized Values for Individual Lymphoid Areas

Source of variation		Degrees of freedom	Mean square
Laboratory chickens			
Total		1072	
Resistant vs. susceptible		1	20.399*
Lines within resistant and susceptible groups		13	2.686
Families within lines		47	2.066†
Individuals within families		131	1.305†
Within individuals		880	.729
Laboratory and Farm chickens			
Total		1420	
Laboratory vs. Farm		1	8.398*
Individuals within Laboratory and Farm		291	1.001
Within individuals		1128	.898
Laboratory resistant, Laboratory susceptible, and Farm			
Total		1420	
Laboratory resistant, Laboratory susceptible, and Farm		2	16.094*
Individuals within groups		290	.922
Within individuals		1128	.899
Means of normalized values			
Resistant lines	1.01		
Susceptible lines	1.31		
Total Laboratory chickens	1.13		
Farm chickens	.95		
All chickens	1.09		

* Significant at the 1 per cent level.
† Significant at the 5 per cent level.

TABLE XXI

Class Frequencies for Number of Lymphoid Areas

Number of lymphoid areas per section	Number of cases		
	Laboratory chickens	Farm chickens	
		A section	B section
0	126	28	32
1	51	17	17
2	36	12	16
3	19	14	8
4	15	6	6
5	13	4	3
6	11	1	8
7	3	2	1
8	10	2	1
9	7	3	
10	3	1	
11	2	2	1
12	1	1	
13	2	1	
14	1		
15			1
16	1	2	1
17	1		
18	2		
19	1		
20	5	1	
21			1
22	1		1
23	2		
24		1	1
25	1	1	
27	2		
28			1
29	1		
34	1		
36	1		
Total	319	99	99

r value is 0.894 (97 degrees of freedom), which is highly significant, thus again indicating that two sections give only a slight increase in accuracy over that obtained with one section. We have yet to learn whether there is a similar degree of correlation between various organs of the body.

TABLE XXII

Average Size of Lymphoid Areas in Laboratory Chickens in Relation to Number per Section

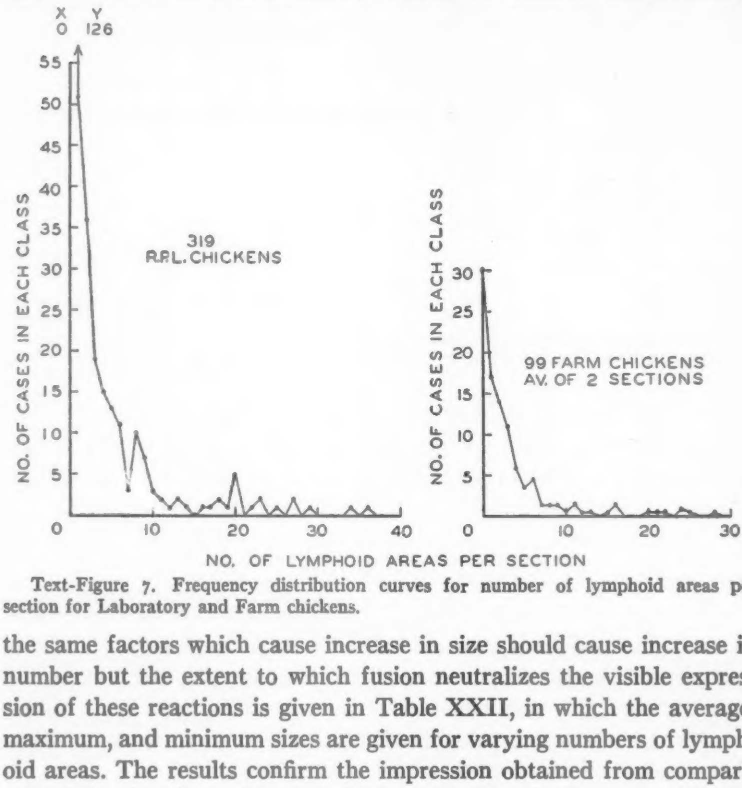
No. of lymphoid areas per section	1	2	3–4	5–6	7–10*	11–20	21–36
No. of cases	51	36	34	24	22	16	9
Minimum (sq. mm.)	.00166	.00424	.00480	.00535	.00895	.01162	.01832
Average (sq. mm.)	.01936	.02070	.02040	.02152	.02366	.04175†	.03693‡
Maximum (sq. mm.)	.05800	.09361	.04852	.04828	.04583	.30404	.10125

* The case shown in Figure 19 of Text-Figure 1 which has 8 lymphoid areas with an average size of 4.46470 sq. mm. was excluded from the above figures.

† If one exceptionally large case is excluded, the average is .02427 and the maximum becomes .04147.

‡ If one exceptionally large case is excluded, the average is .02889 and the maximum becomes .05386.

The chief difference between the curves for number (Text-Figure 7) and those previously given for percentage and size (Text-Figures 2 and 6) is the absence of a greatly extended tail along the horizontal axis. This is probably due to the fact that individual areas close together fuse as they enlarge and thereby reduce the number. Theoretically,



Text-Figure 7. Frequency distribution curves for number of lymphoid areas per section for Laboratory and Farm chickens.

the same factors which cause increase in size should cause increase in number but the extent to which fusion neutralizes the visible expression of these reactions is given in Table XXII, in which the average, maximum, and minimum sizes are given for varying numbers of lymphoid areas. The results confirm the impression obtained from comparison of the drawings. In general the interacting factors are such that the average size of the lymphoid area does not show a great increase with number; however, there is a slight but steady progression in that direction and also for the maximum and minimum values.

Similar data were not collected for Farm birds because the number of cases was too small.

Discussion

When brought together, the principal facts in this and the previous paper[1] should reveal some information about how the agent of lymphomatosis acts. In the first article the character of the lymphoid

tissue in relation to amount in the section and to size of the individual area was considered. It was concluded that no fundamental histologic differences could be distinguished regardless of size or amount until the area was occupied by wildly proliferative large lymphoid cells. It was seriously questioned whether any lymphoid tissue could be considered normal in the sense that lymph nodes of mammals are normal. Much of the lymphoid tissue as it grew destroyed adjacent pancreatic tissue and some of it apparently developed by plugging small blood vessels.

The quantitative analysis has shown a significant correlation between the amount of lymphoid tissue in survivors of families and lines of chickens and the percentage of lymphomatosis deaths for the respective groups. The conclusion seems justified on the basis of these data that the sum total of lymphoid reactions reflects the action of the disease agent. Resistance and susceptibility of the host play a rôle. Not only was the percentage of lymphoid tissue less, but the average size of individual lymphoid areas was less for resistant lines than for susceptible lines. If the dose factor of the agent was the same for all Laboratory chickens, it would appear that resistant birds inhibited the action of the agent either by providing an unsuitable habitat for its multiplication or by exerting a greater inhibition against uncontrolled growth. Perhaps both factors are involved.

Farm birds prepared for market were similarly analyzed. A comparison of them with Laboratory birds showed that the percentage of lymphoid tissue was not significantly different. Further similar studies should be made on other flocks to determine the significance of these results. The evidence thus far shows that the Farm chickens are intermediate between the Laboratory birds when the latter are divided into resistant and susceptible groups. The data are not yet sufficient for one to use the quantity of lymphoid tissue for estimation of the amount of potential lymphomatosis in a farm flock, chiefly because age and breed are unknown variables and we do not yet have a measure of lymphoid reaction to common infectious diseases of poultry. Some preliminary information was obtained along these lines but more is needed. Also, more information is needed concerning the effects when varying amounts of the agent are acquired by natural means of transmission. The size of individual lymphoid areas was significantly less for Farm chickens than for Laboratory chickens.

The remarkably constant rate of death of birds showing grossly visible tumors, especially between 200 and 600 days, was brought out in Table VI. The maximum deviation for each 100-day period varied

only from 12 to 12.6 per cent. Since this rate is so constant, in birds killed at the same age one should see an approximate picture of the pathogenesis of this disease, at least as it applies to the flock as a whole. Such a procedure is one commonly adopted for estimating the relative duration of different phases of the mitotic cycle[9,10] in which the assumption is employed that those phases occurring most frequently will be those which, on the average, have the longest duration. Just as there are fluctuations between the rate of mitosis of individual cells, so there will be differences between individual chickens.

Growth is cumulative and the cumulative picture of lymphoid tissue at the termination date is shown in Text-Figure 3. Ordinates of "time" and "size of tumor" are suggested. Time has been considered equivalent to incidence on the basis, already mentioned, that the rate of development in the flock is constant. About 90 per cent of the time is involved in developing a tumor which in area is 1 per cent of the section area. On a volume basis an even greater proportion of time will be required to reach the 1 per cent level. From that point on, development proceeds with great rapidity and the tumor will soon attain a many-fold increase in size to the point where it becomes grossly visible. If a hyperbolic curve accurately portrays the pathogenesis in the population, presumably the individual follows a similar curve; but the equation for the hyperbola will vary for each individual, so that in a bird which dies early the focal point of the curve is probably further removed from the point of axial intersection than in a bird which develops a tumor late in life.

Thus far only a relative time scale has been considered. The data can be used to gain some idea of the absolute time scale. Since the rate of visible tumor formation associated with death is 12 per cent for each 100 days and at termination 4 per cent of the population was positive for lymphomatosis, about 33 days is required for the development of the grossly visible tumor. This again is an average figure for the population and there were about twice as many resistant birds as susceptible ones, so that the figure of 33 days is probably a little high. Tumor development probably would be more rapid in young, susceptible birds. Davis and Doyle,[11] making use of biopsies, reported "fatal cases of visceral lymphomatosis developed very rapidly; in some cases, which showed definite liver lesions at postmortem examination, death occurred in three or four weeks after biopsy had showed the liver to be apparently free of an abnormal amount of lymphoid infiltration." The two approaches to this problem give results in close agreement.

The problem of separating so-called normal from lymphomatous lesions has long been recognized.[12-14] Thus far, however, no one has clearly set forth criteria which even arbitrarily would permit a uniform microscopic diagnosis of positive or negative for this disease. The conclusion has been presented in this study that all lymphoid accumulations in a parenchymal organ such as the pancreas are probably abnormal. On the other hand, we definitely do not suggest that all ectopic lymphoid areas are specific for this disease, nor is it claimed that small amounts of lymphoid tissue are not compatible with life, for lymphoid tissues undoubtedly play a part in the defense mechanisms of the body. Therefore, to be relatively safe in making a diagnosis for practical purposes, the separation of microscopically positive and negative should be made somewhere beyond the point of greatest curvature (Text-Fig. 2). As a purely arbitrary point, probably 1 per cent lymphoid tissue is a workable value. On the same basis any lymphoid area larger than 0.1 sq. mm. is suggestive of lymphomatosis. It would not be desirable to base a diagnosis on number of lymphoid areas alone, since this variable is influenced by fusion as the areas increase in size. If a 1 per cent level is chosen, for the Laboratory birds 35, or 11 per cent, of the pancreases would be diagnosed as positive. It should be noted that this value is only slightly less than the 12 per cent of chickens (Table VI) which presumably would die in the succeeding 100 days with grossly visible lymphoid tumors. On the basis of containing at least one lymphoid area above 0.1 sq. mm., there are 19 cases, or 6 per cent. Of these, 14 had lymphoid tissue above 1 per cent; 5 of them had less.

Of the two methods, very probably the percentage of lymphoid tissue gives the more accurate measure; the second criterion misses too many cases with large amounts of lymphoid tissue. The chief value in using size of a lymphoid area would be to decide between borderline cases. These criteria have been set up for the pancreas; whether they are applicable to the liver is not known but material has been collected for this additional study. If the 1 per cent level is applied to the Farm birds (both sections), the microscopically positive cases are about 5 per cent, which is less than for the Laboratory birds.

A single section undoubtedly misses many cases which should be called positive microscopically. It is questionable whether any chicken is completely free of ectopic lymphoid areas, so that if sections from ten or more regions were examined it is probable that the zero class in the percentage curve would be nearly eliminated, but that in no wise automatically changes the region of greatest curvature when compari-

sons are made using the same units on the axes. The sample is considered adequate to serve as a measure of Laboratory chickens in that over one-fourth of those hatched in 1945 for genetic studies was used for the histologic study and these are distributed among 14 inbred populations and a cross. Any population of birds would probably give distribution curves of approximately the same general shape as those obtained with this material, but the position of the curves in relation to the axes might shift for different groups and perhaps for different ages.

When it is considered that the 1 per cent level is based on a population having a high incidence of lymphomatosis in which no culling took place, it is our opinion that this suggested arbitrary level is reasonably conservative. When further information is obtained concerning the action of the naturally occurring agent or agents on the development of lymphoid tissue, it will probably be possible to modify this level and make it more exact.

It is evident from the right-hand curve in Text-Figure 4 that some birds diagnosed as grossly positive for lymphoid tumors did not show lymphoid tissue in the section. It has been mentioned already that a single section is probably not an adequate sample for an individual bird but it is regarded as an adequate sample when studying a large population. A further explanation of this apparent lack of correlation between gross and microscopic findings is evident when one recalls the discussion of Text-Figure 3. There are bound to be slight inequalities in rate of growth of individual lymphoid areas; that is evident from the wide range in size observed in a single section. Such fluctuations in size and rate of growth have little significance when one is dealing with that portion of the curve which approaches the vertical asymptote, but when a particular lymphoid area passes the point of greatest curvature, its change in size in relation to time has an entirely different relationship, and a lymphoid tumor slightly ahead in its development would grow rapidly to visible size, whereas others on the same time-growth scale might still be less than the 1 per cent level. The size difference becomes great but the time difference is small.

Undoubtedly the microscopic analysis of one section, or of a few sections, misses some positive cases; however, some positive cases are found by microscopic examination which are missed in the gross examination. Neither method is complete in itself and each should be used to supplement the other. The final tabulation for the Laboratory chickens would then be:

Grossly positive and microscopically positive	3
Grossly positive and microscopically negative	11
Grossly negative and microscopically positive	33
Total	47 or 14.7 per cent of the population examined

This is over three times the incidence based on gross diagnosis alone and perhaps would be higher if other organs were included in the examination.

It is well known that ducks and other birds, in which no grossly visible lymphoid tumors have been reported, have ectopic lymphoid areas. Material has been collected from ducks, both wild and domestic,[16] turkeys,[15] pheasants, pigeons, and doves, as well as from numerous species of wild birds. It is planned to present the results in later publications.

Summary

In studying a population of common fowls, two methods have been evaluated for comparing the variables: deaths with lymphoid tumors, and deaths associated with other causes. Method II, in which the population was taken as deaths due to one or the other variable alone plus survivors, is regarded as the better for measurement of performance.

Incidence of deaths with lymphoid tumors compared with deaths from other causes shows a correlation for genetic lines beyond the 1 per cent level.

In a population of chickens, either Laboratory or Farm stock, the lymphoid areas in the pancreas are distributed in J-shaped curves when based on one or two sections from the organ. This is true for percentage of lymphoid tissue, the size of individual lymphoid areas, and the number of lymphoid areas.

For Laboratory chickens there are significant correlations between the lymphoid tissues in survivors which are largely diagnosed as lymphomatosis negative, and the percentage of deaths due to the disease preceding the termination date. Analysis on the basis of lines gave a higher correlation than on the basis of families.

In resistant lines not only was the percentage of lymphoid tissue less but also the individual lymphoid areas were smaller.

Analysis of variance of coded values for percentage of lymphoid tissue revealed no significant difference between the Laboratory birds

as a whole and the Farm birds, but there were highly significant differences when the Laboratory chickens were divided into a resistant and a susceptible group. The Farm group was intermediate. Analysis of coded values for size of individual lymphoid areas revealed that the mean values for resistant Laboratory chickens and Farm chickens were similar and significantly different from susceptible Laboratory birds.

On the basis of a previous paper as well as this one, it is concluded that small lymphoid areas are probably not normal for an organ such as the pancreas. At present we do not know how specific such lymphoid areas are for the agent of lymphomatosis, just as we do not know whether all grossly visible neoplastic lymphomata are due to the agent of lymphomatosis.

Until the early stages in the pathogen-host interactions have been worked out, a tentative and purely arbitrary basis for microscopic diagnosis has been presented on the basis of distribution curves thus far obtained. It is suggested that values of about 1 per cent lymphoid tissue for the pancreas should be regarded as microscopically positive for lymphomatosis. A less exact measure is the size of individual lymphoid areas, but when they exceed 0.1 sq. mm. they can be of value in deciding borderline cases.

A rough measure of pathogenesis can be obtained from the data. The growth of lymphoid tissue appears to follow a hyperbolic curve in which a large proportion of the total time is involved in development up to the 1 per cent level and after that point the rate is rapidly accelerated. Supporting evidence comes from the data on grossly visible tumors in which it is calculated that on the average only about 33 days are required to develop such tumors after the stage at which they just escape recognition at gross examination.

We wish to acknowledge the technical assistance of Mrs. Janet B. Breitmayer.

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SKELETAL GROWTH AND ARTICULAR CHANGES IN MICE RECEIVING A HIGH-FAT DIET*

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In young guinea-pigs and rats, underfeeding delayed skeletal development.[1,2] Conversely, growth and ageing of cartilage and bone progressed more rapidly in animals receiving dietary supplements of milk and liver than in those fed chiefly carbohydrates.[2-4] These findings suggested an inquiry into the influence of individual dietary constituents on the skeletal time curve.

The present report deals with observations on the effect of a high-fat diet on skeletal growth and development of young mice.

Material and Methods

Sixty male mice of the closely inbred strain C57 black were at the time of weaning divided into two groups: Thirty control animals were kept on a stock diet of Ralston purina laboratory chow no. B-2362. This ration is composed as follows:

Moisture	Per cent	8.90
Protein	Per cent	26.18
Fat	Per cent	5.35
Fiber	Per cent	4.62
Ash	Per cent	6.49
Nitrogen-free extract	Per cent	48.46
Calcium	Per cent	1.17
Phosphorus	Per cent	0.87
Magnesium	Per cent	0.196
Iron	Parts per million	327.0
Manganese	Parts per million	106.0
Copper	Parts per million	16.8
Cobalt	Parts per million	0.14
Potassium	Per cent	0.90
Carotene	Parts per million	6.6
Thiamin	Parts per million	12.85
Riboflavin	Parts per million	7.49
Niacin	Parts per million	65.6
Vitamin D U.S.P.	Units per gm.	4.97
Vitamin A U.S.P.	Units per gm.	9.0

Thirty animals received this ration ground to a meal with 25 per cent lard (Swift's "silverleaf") added. Balls formed from the thoroughly mixed lard and meal were fed *ad libitum*. Weights of the mice

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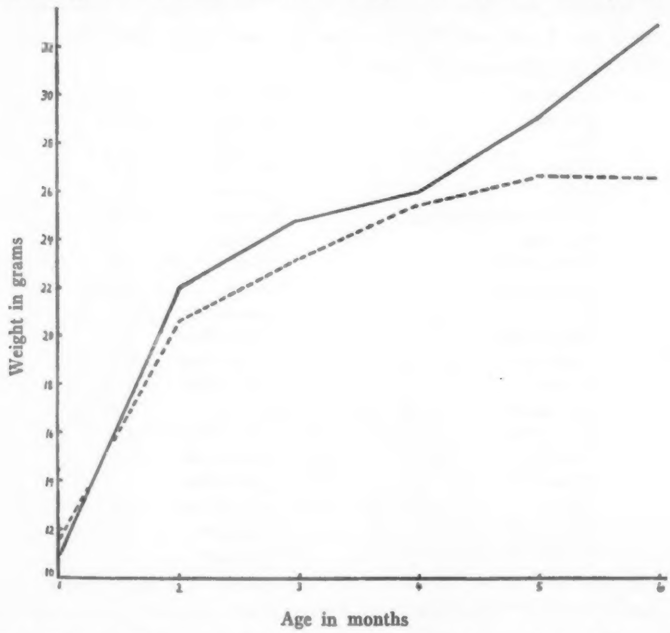
were taken once a week until they had reached 25 gm. Thereafter, they were weighed once a month.

The experimental mice and their controls were sacrificed at the age of 1½, 2, 3, 4, and 6 months. The test animals were thus kept on the high-fat diet for ½, 1, 2, 3, or 5 months. At necropsy, tibiae and femora were removed as a whole, fixed in 4 per cent formaldehyde, and decalcified in 5 per cent nitric acid. One leg, including the knee-joint and patella, was embedded in paraffin; semi-serial sections were stained with hematoxylin and eosin. The other leg was saved for cytochemical studies.

Gross Observations

The animals tolerated the high-fat diet well. After about 1 month, the fur became greasy but otherwise the mice appeared healthy throughout the period of observation.

Text-Figure 1 shows the mean weights of 87 test and 71 control



Text-Figure 1. The mean weights of 87 test animals and 71 control animals were used in preparing these curves. The solid line was derived from those on the high-fat diet; the broken line, from those on the control diet.

animals. Of these, 57 test and 41 control animals are still under observation.

The mean weights of the animals, and the maximum and minimum deviations from these means, were as follows:

Age of animals	Control animals	Test animals
	grams	*grams*
1 month (initial)	11.6 Max. d., 2.4; min. d., 0.6	10.9 Max. d., 3.1; min. d., 2.4
2 months	20.6 Max. d., 2.4; min. d., 2.1	21.9 Max. d., 2.6; min. d., 3.9
3 months	23.2 Max. d., 4.3; min. d., 1.2	24.7 Max. d., 5.3; min. d., 5.2
4 months	25.5 Max. d., 1.5; min. d., 0.5	25.9 Max. d., 6.6; min. d., 3.4
5 months	26.7 Max. d., 2.3; min. d., 1.7	29.0 Max. d., 10.0; min. d., 3.5
6 months	26.6 Max. d., 3.4; min. d., 1.6	33.3 Max. d., 4.2; min. d., 6.8

The difference in the two weight curves became conspicuous during the fifth month of age. At that time, when the control mice had reached a certain plateau, the animals kept on the high-fat diet continued to gain weight. Considerable variations in the response to the high-fat diet were indicated by the wide deviations from the mean weights. For the present investigation, animals were chosen whose weights were in the lower range.

Histologic Examination

Unless otherwise mentioned, the following description is based on the findings in the growth zones at the upper end of the tibia. Measurements and counts represent averages obtained from the examination of all animals in each group. A more detailed account of the normal structure of the growth zones and of the skeletal time curve has been given previously.[5]

GROWTH ZONES

Mice One and One-Half Months Old

Animals Fed the Control Diet. The epiphyseal disks were 260 μ wide. The cartilage cell rows were in a regular configuration and were separated from each other by thin layers of chondromucoid ground substance. The single row consisted of 10 to 12 columnar and 4 or 5 hypertrophic cells. Mitotic figures were frequent. The replacement of the hypertrophic cartilage by bone was progressing. The metaphysis contained numerous thin-walled capillaries surrounded by a loose connective tissue. The osseous spicules were delicate and covered by osteoblasts; osteoclasts were scarce. The shaft contained large osteocytes.

Animals Fed the High-Fat Diet for One-Half Month. The growth zones measured 235 μ, and their structure differed only slightly from that seen in the control animals. The individual cartilage cell row consisted of 8 to 10 columnar and 4 or 5 hypertrophic cells. The colum-

nar cells began to enlarge more proximally in the growth zone than in the controls. The conversion of columnar into hypertrophic cartilage cells was thus accelerated. Calcification of the cartilage was more marked, the matrix was more abundant, and the replacement of cartilage by bone was more accentuated than ordinarily. The metaphyseal spicules were thick and covered by many large osteoblasts. The trabeculae as well as the compact bone of the shaft were composed of small osteocytes and much ground substance.

Mice Two Months Old

Animals Fed the Control Diet. The arrangement of the cartilage cell rows was regular but there was more matrix than in the younger control group. The epiphyseal plates were 155 μ wide (Fig. 1). The single row consisted of 8 or 9 columnar and 3 or 4 hypertrophic cartilage cells; mitotic figures were still numerous. The metaphyseal trabeculae were longer and more cellular, and the cortex of the shaft was thicker than at the earlier age.

Animals Fed the High-Fat Diet for One Month. The zones of endochondral ossification were narrowed to 110 μ (Fig. 2). There were fewer and shorter cartilage cell rows than in the animals fed the control diet; the rows were composed of only 5 or 6 columnar and 2 or 3 hypertrophic cells. The proliferation of the cartilage was distinctly decreased, but again, hypertrophy was observed to set in more proximally than in the controls. Large amounts of dense hyalinized ground substance appeared between the cartilage cell rows. Much calcium was deposited at the periphery of the cartilage cells so that the individual cells stood out prominently. Single cartilage cells and entire cartilage cell rows had been replaced by small or medium-sized hyalinized or ossified plugs of intercellular substance. In the femur, these plugs were thicker and more numerous than in the tibia. The metaphysis was vascular; numerous large osteoblasts and much metaphyseal bone were present. The spicules were broad-based, thick and short, and showed some transverse links. The osteocytes were smaller, and the bony substance was more compact than in the control animals. The cortex of the shaft was thicker, and osteoblasts as well as bone cells were more numerous than ordinarily.

Mice Three Months Old

Animals Fed the Control Diet. The epiphyseal plates measured 120 μ (Fig. 3). The cartilage cell rows were less numerous, and the ground substance was more abundant and more hyalinized than in

the younger control groups. The growth processes in the cartilage had declined; both types of cartilage cells were smaller than at the earlier ages, and the single cartilage cell row contained only 7 or 8 columnar and 3 hypertrophic cells. Some cells were completely sclerosed, and in one mouse an occasional cell row was broken down and replaced by a narrow bony plug. However, in the other animals of this group no such regressive changes had occurred. The metaphyseal spicules and the cortex were thicker than in the younger mice.

Animals Fed the High-Fat Diet for Two Months. The epiphyseal plates were in some places narrowed to 80 μ, but they had an over-all width of about 100 μ. The heavily calcified cartilage cell rows contained 5 or 6 columnar and 1 or 2 sclerosed hypertrophic cells. The difference between these two types had become less distinct. No new columnar cells were produced, and the old ones had undergone hypertrophy. Many thick osseous plugs had replaced the destroyed cartilage cell rows. These bony plugs (Fig. 4) were, in some places, perforated by vessels indicating impending epiphyseo-diaphyseal union. In 2 of these mice, wider gaps were observed in the femur. The trabeculae were less regular than usual. They were coarse, contained much calcium, and formed many thick interlacing bridges. Osteoblasts were numerous. Underneath the hypertrophic cartilage, a transverse bony lamella was being laid down. The compacta of the shaft was thick and contained distinct cement lines and many small osteocytes.

Mice Four Months Old

Animals Fed the Control Diet. The epiphyseal disks were 85 μ wide (Fig. 5). Mitotic proliferation of the columnar cartilage had ceased. The single cell row was composed of 6 to 8 columnar and 2 or 3 small cells of hypertrophic type. The cell counts were thus similar to those seen in the control mice 3 months of age, but the cartilage cells were more closely packed than before. This was due to a marked consolidation and shrinkage of the ground substance. Between the cartilage columns small plugs of sclerosed, calcified, or ossified material appeared. The primary spongiosa was thick and contained much calcium. Calcification of the compacta of the shaft was likewise more prominent than in the younger age groups.

Animals Fed the High-Fat Diet for Three Months. The width of the epiphyseal plates varied between 65 and 80 μ (Fig. 6). Where cell counts could still be made, the single row was composed of 4 or 5 small columnar and 1 or 2 hyalinized hypertrophic cells. More frequently, however, calcification and ossification were so advanced as to make a

cell count impossible. Amorphous or ossified plugs were common, and they obliterated the cellular architecture of the growth zones. Some capillaries advanced from the metaphysis, resorbed these plugs, and thus initiated perforations of the epiphyseal plates. As usual, epiphyseo-diaphyseal union was farther advanced in the femur than in the tibia. The inactive cartilage was delimited from the bone marrow by a transverse, discontinuous, osseous lamella of irregular thickness. Most of the primary spongiosa had been resorbed, and here and there only scattered fragments of spicules were seen in the metaphysis. The shaft was thick and solid.

Mice Six Months Old

Animals Fed the Control Diet. On the whole, the epiphyseal plates were narrower than before, and they were too irregular to be measured. Neither could accurate cell counts be made. There was loss of cellularity and a marked increase of hyalinized and calcified intercellular substance (Fig. 7). Large amorphous or bony plugs traversed the growth zones. In none of the mice had epiphyseo-diaphyseal union been initiated in either tibia or femur. The cartilage was delimited from the metaphyseal marrow by a moderately thick discontinuous osseous plate. The trabeculae were short and scanty, and the cortical bone was thick and compact.

Animals Fed the High-Fat Diet for Five Months. The epiphyseal plates were represented by a discontinuous irregular band of hyalinized and calcified cartilage in which a columnar arrangement was only faintly recognizable (Fig. 8). Plugs consisting of amorphous or ossified material were numerous; many were perforated by vessels, and break-through had occurred or was about to occur in both tibia and femur. The bony lamella underneath the inactive cartilage was thicker and coarser than in the corresponding controls. The cortex of the shaft had not changed appreciably.

JOINTS

Animals Fed the Control Diet. Throughout the period of observation, the articular surfaces showed an essentially uniform architecture (Fig. 9). Three layers of cells could be distinguished: The uppermost, the sliding zone, contained flat spindle-shaped cells. The underlying transitional zone showed round cells with small nuclei and larger amounts of clear cytoplasm often grouped together in twos or threes. In the layer adjoining the bone, the cartilage cells underwent hypertrophy; they became replaced by bone which formed a thick lamella and some coarse trabeculae. With increasing age, the hypertrophy of

the cartilage became more accentuated and extended into the transitional zone. In mice 6 months of age, there were foci of hyperplasia in addition. Sometimes two or three cartilage cells were surrounded by a dark basophilic capsule. At the same time, single cartilage cells or small groups of cells had undergone regression. They were swollen or liquefied, and the outlines of their capsules became indistinct. In one of 6 mice examined at the age of 6 months, these changes were present also in the patella.

The inner synovial layer was cellular, not adherent to the articular cartilage, and in many places covered by regular lining cells; the outer synovial layer consisted of dense collagenous tissue. With advancing age, the synovial membranes formed vascular villous projections. The interarticular ligaments were composed of dense fibrous tissue interspersed with some islands of precartilage.

Animals Fed the High-Fat Diet. The changes taking place in the different tissues of the knee joint will be described separately. This does not mean, however, that they always occurred as isolated lesions. All combinations were observed. As a rule, the older the animal, the more advanced and extensive were the changes, and the more tissues were involved. Altogether, 12 of 18 mice 3 months of age and older showed these combinations. The pathologic changes may be grouped as follows:

In the cartilage, hyperplasia and hypertrophy were noted as early as after 1 month but more pronouncedly after 2 months of feeding the high-fat diet. Commonly, the hypertrophic processes preceded the hyperplastic ones. An increased number of enlarged cartilage cells and incubator capsules were observed in the articular surfaces of tibia, femur, and patella (Figs. 10 and 12). These changes varied in degree and were patchy in distribution. Some areas showed hyperplasia, others hypertrophy, and in still others the cartilage was not remarkable. The earliest lesions were usually located in the more anterior joint surfaces, particularly near the insertion of the ligaments. At first the lesion was found in only one bone, but later both the long bones and the patella were affected. These alterations were present in 21 of 24 mice 2 months of age and older.

The type, degree, and distribution of regressive changes varied from animal to animal. They were often found in the patella before they appeared in other parts of the joint. Vacuolation of cells and matrix and deposition of fine basophilic granules were early manifestations of degenerative processes. More severe injury was indicated by fibrillation, swelling, myxoid and fibrinoid degeneration, or liquefaction of

the cartilage (Figs. 11, 14, 15, 17, and 18). Fragments of cartilage were broken off and came to lie free in the joint cavity. Such changes were noted in 19 of 24 mice 2 months of age and older.

In the ligaments, the connective tissue had undergone myxoid or hyaline change. The precartilage became converted into true hyaline cartilage which formed small islands within the substance of the ligaments. This cartilage in turn had undergone regression. Of a total of 24 mice over the age of 2 months, the joints of 15 animals were thus affected.

In the synovialis, edema and myxoid change of the connective tissue were the earliest indications of disease. The loose areolar tissue had begun to proliferate and penetrated the articular surface, usually following the nutrient vessels (Figs. 13 and 14). Thus it grew into the marrow cavity and replaced parts of the hemopoietic marrow. In advanced cases, the synovial tissue spread in a pannus-like fashion over the surfaces of the joint and the ligaments (Fig. 17). The synovial fat pads increased in size and protruded farther than ordinarily into the joint cavity. These changes were present in 8 of 18 mice sacrificed at 3 months of age and later.

The bone underlying the articular cartilage was rarefied, in particular near the insertion of the ligaments and in areas in which the cartilaginous covering had been thinned out or eroded. The preserved bone, however, showed no evidence of demineralization (Fig. 14). In 5 of 12 mice over 4 months of age, atrophy of the bone was advanced.

In the epiphyseal bone marrow, focal fibrosis and myxoid degeneration or small hemorrhages with deposition of blood pigment and formation of small cysts were noted (Fig. 16). These changes were present in 7 of 12 mice 4 months of age and older.

Discussion

In growing male mice of strain C57 black, skeletal growth and development were markedly accelerated by feeding a diet containing 25 per cent lard in addition to the 5.35 per cent fat present in the control diet. During the first 4 months of observation, the animals fed the high-fat diet gained slightly more weight than the controls. However, considerable differences existed in the response of the individual animals. From the age of 5 months on, the mean weights of the test animals exceeded those of the controls more and more, and at the age of 6 months, the weights of the mice kept on the high-fat diet surpassed those of their controls by as much as 25 per cent. In regard to their weights, mice thus respond much like rats reared on a high-fat diet.[6,7]

In the growth zones of mice fed the high-fat diet, hypertrophy and hyperplasia of the cartilage were increased, the primary spongiosa was coarser, and the osseous substance in metaphysis and shaft was more abundant than in the control animals. Degeneration and calcification of the cartilage were accelerated and intensified. Perforations of the epiphyseal plates by vessels, the forerunners of epiphyseo-diaphyseal union, were initiated at a much earlier age than ordinarily. There were differences in the response of the individual animals. As a rule, however, the growth zones of mice 2 months of age and fed the high-fat diet for 1 month (Fig. 2) resembled those of control animals 4 months of age (Fig. 5). In 3-months-old mice fed the high-fat diet, the growth zones contained large osseous plugs; in control animals, comparable conditions were found at ½ year of age or later. The primary spongiosa, ordinarily not resorbed before the age of 6 months (Fig. 7), had, under the influence of the high-fat diet, been dissolved at 4 months of age (Fig. 6).

In the articular cartilage, the high-fat diet led to stimulation of growth and an accentuation of regressive changes. The changes thus produced constitute no mere acceleration of physiologic processes as in the growth zones; they do not occur under normal conditions and have thus to be considered as pathologic lesions, comparable to human osteo-arthritis. During the stages under consideration, marginal outgrowth of bone was not seen; there was in some cases an atrophy of the bone underlying the articular cartilage. This may be due to the fact that the present experiments were terminated at the comparatively early age of 6 months. However, there is also the possibility that some mice tend to develop atrophic rather than hypertrophic articular disease. Long-stage experiments are in progress to determine the further course of these joint changes.

Degenerative articular lesions occur spontaneously in ageing mice.[5] Considerable strain differences exist in the time of onset and in the severity and incidence of these lesions. Sex and breeding also play a rôle in the susceptibility of the animals. The changes in the joints developing under the influence of the high-fat diet were morphologically indistinguishable from the spontaneous lesions and from those induced by the administration of anterior hypophyseal hormone. The question whether this morphologic similarity is more than a mere coincidence is being investigated. There may be a closer relationship between these two forms of articular lesions. This supposition is strengthened by recent observations concerning the influence of an anterior hypophyseal hormone on the fat metabolism of mice.[8]

The overweight of the animals might be considered a contributing factor in the origin of articular lesions inasmuch as it increases the mechanical stress on the joints. However, joint lesions were present at the age of 2 or 3 months, at a time when the difference in the weights of the test and control mice was not very marked. Moreover, in order to avoid as much as possible a complicating effect of overweight, animals in the lower weight ranges were chosen for the present study. In addition, mechanical stress applied to the joints of ageing mice did not alter the course of spontaneous articular lesions.[9] Therefore, it is not unlikely that, as has been claimed for human osteo-arthritis, metabolic factors are involved in the production of joint disease of this type in mice. Whether under the influence of the high-fat diet the chemical composition of the cartilage itself changes, is being studied.

Summary

In growing male mice of strain C57 black, observed from the time of weaning to the age of 6 months, a diet containing 25 per cent lard accelerated skeletal growth and development. In the articular cartilage, changes comparable to early degenerative joint disease of man were produced. These lesions were morphologically indistinguishable from the articular changes occurring spontaneously in old mice.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

Figures 1 to 8 represent sections of the growth zones at the upper end of the tibia of male mice of strain C57 black.

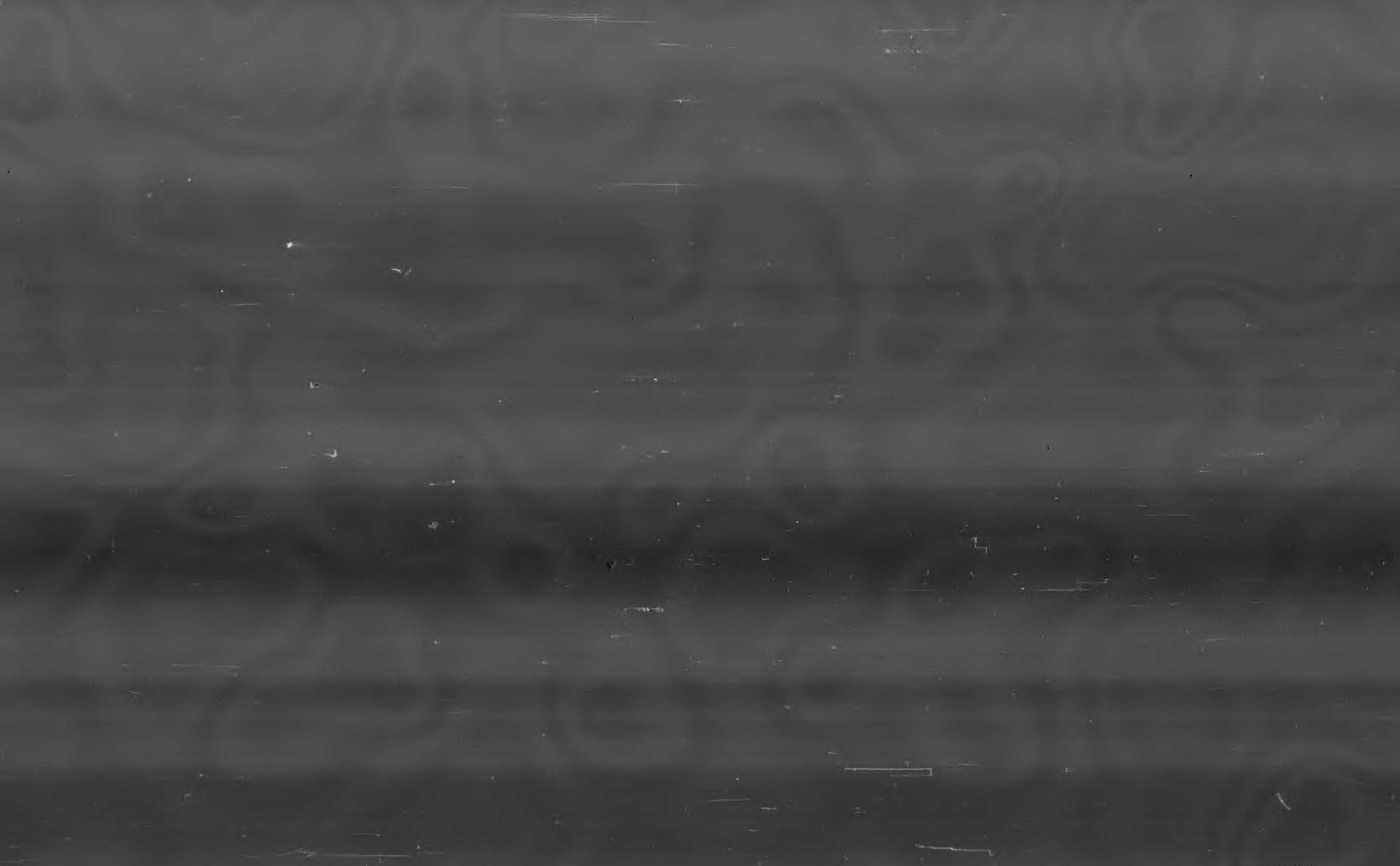
PLATE 14

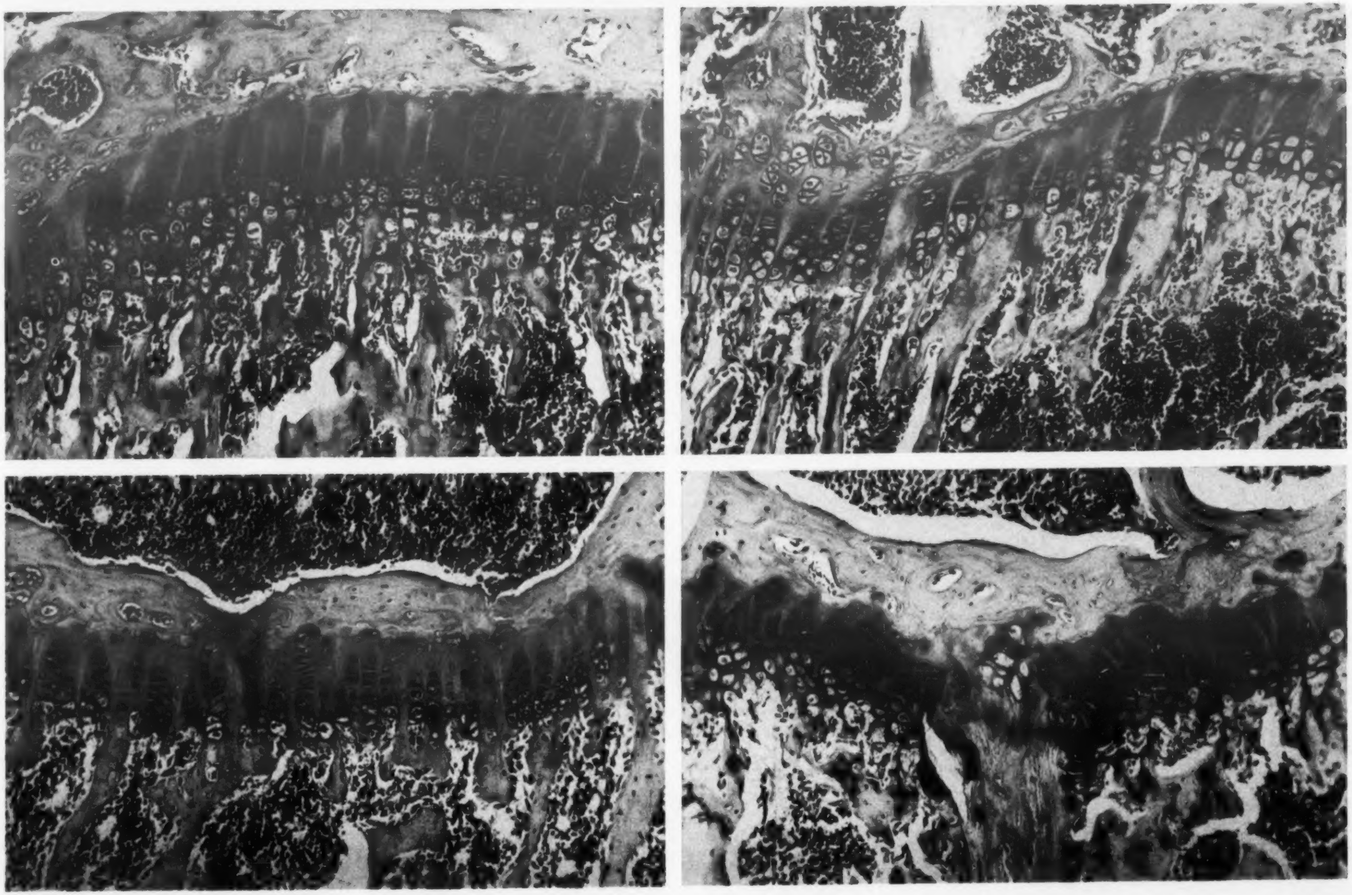
FIG. 1. Control mouse, 2 months old. Cartilage and primary spongiosa show regular configuration. × 150.

FIG. 2. Mouse, 2 months old, fed the high-fat diet for 1 month. The cartilage cells are decreased in number; hypertrophy sets in more proximally, the cartilaginous matrix is more abundant, and the trabeculae are shorter and thicker than in the control (Fig. 1). × 150.

FIG. 3. Control mouse, 3 months old. The epiphyseal plate is narrower, the cells are less numerous and the matrix is more abundant, the spicules are shorter and thicker than in the 2-months-old control (Fig. 1). × 150.

FIG. 4. Three-months-old mouse, fed the high-fat diet for 2 months. The epiphyseal plate is narrower than in the control (Fig. 3). A large osseous plug has replaced an area of cartilage. The spicules are short, thick, and begin to show transverse links. × 150.





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Skeletal Changes with a High-Fat Diet

Plate 15

Fig. 5. Control mouse, 4 months old. The epiphyseal plate is further narrowed and more intensely calcified, the matrix is denser, and the primary spicules are longer and thicker than in the younger controls (Figs. 1 and 3). × 150.

Fig. 6. Four-months-old mouse, fed the high-fat diet for 3 months. The epiphyseal plate is irregular in width. The number of cells is decreased, and numerous plugs have replaced the cartilage. The primary spongiosa has been resorbed, and a transverse osseous lamella is seen below the inactive cartilage. × 150.

Fig. 7. Control mouse, 6-months old. The epiphyseal plate resembles that of a 4-months-old animal fed the high-fat diet for 3 months (Fig. 6). × 150.

Fig. 8. Six-months-old mouse, fed the high-fat diet for 5 months. The cartilage is very irregular, calcified and ossified. A large osseous plug with a small perforating blood vessel is seen in the center. The subepiphyseal bony lamella is thick. × 150.

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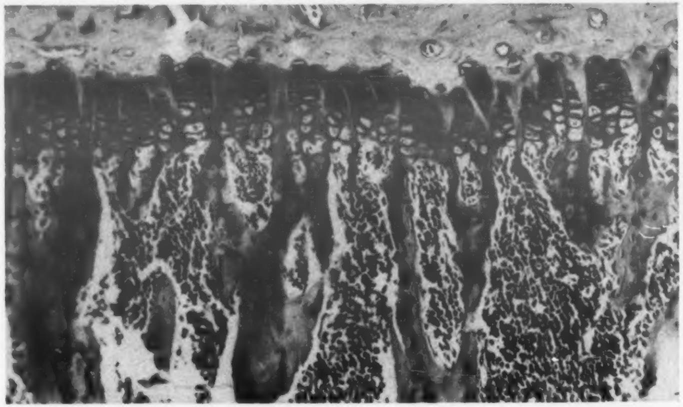

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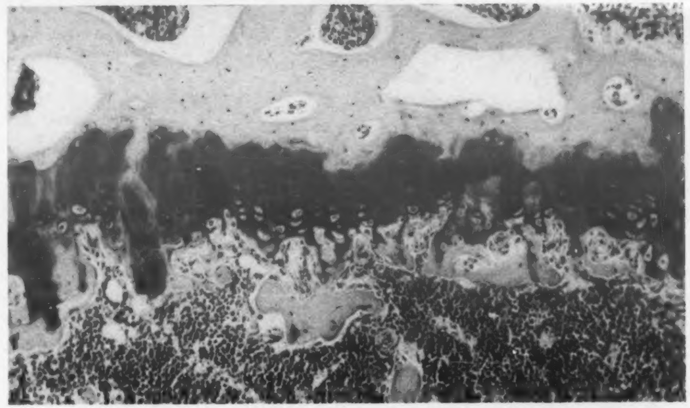

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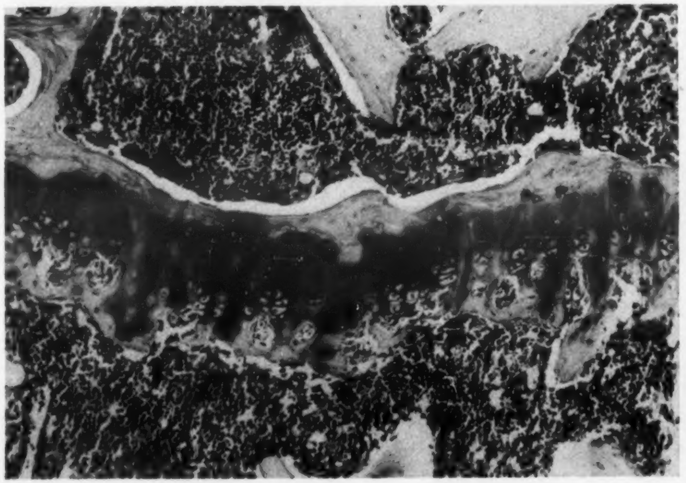

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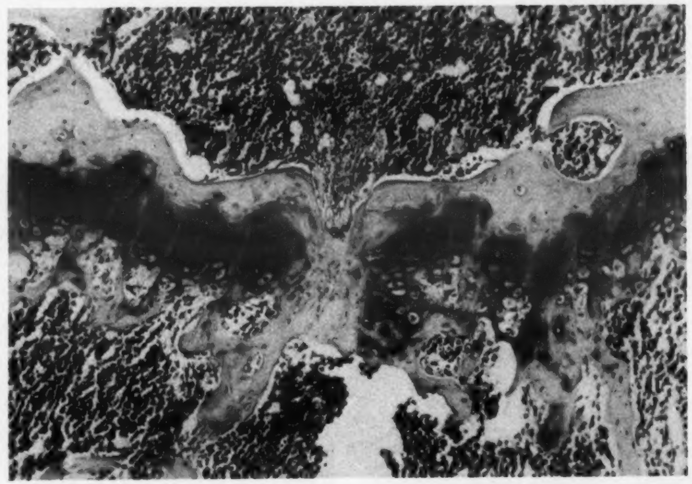

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Skeletal Changes with a High-Fat Diet

Plate 16

Figures 9 to 13 represent sections through the knee joint of male mice of strain C57 black.

Fig. 9. Control mouse, 6 months old. Articular surface of the tibia. The cartilage cells increase in size from the surface towards the epiphyseal marrow. × 225.

Fig. 10. Two-months-old mouse, fed the high-fat diet for 1 month. Articular surface of the tibia. Two foci of hypertrophy and hyperplasia of the cartilage with calcification and fibrillation of the matrix are seen. × 225.

Fig. 11. Six-months-old mouse, fed the high-fat diet for 5 months. Cartilage of the patella showing a focus of liquefaction. × 225.

Fig. 12. Six-months-old mouse, fed the high-fat diet for 5 months. Articular surface of the tibia. Hypertrophy and hyperplasia of the cartilage cells and heavy basophilia of the cell capsules are observed. × 225.

Fig. 13. Six-months-old mouse, fed the high-fat diet for 5 months. Articular surface of the tibia. A broad band of synovial tissue penetrates into the epiphyseal marrow cavity. × 225.

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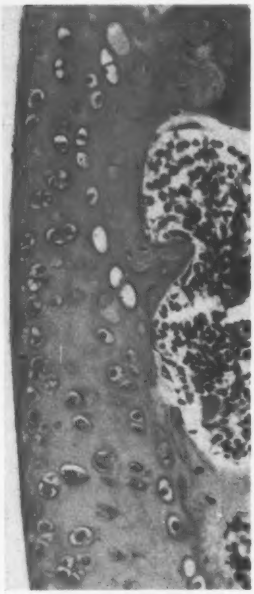

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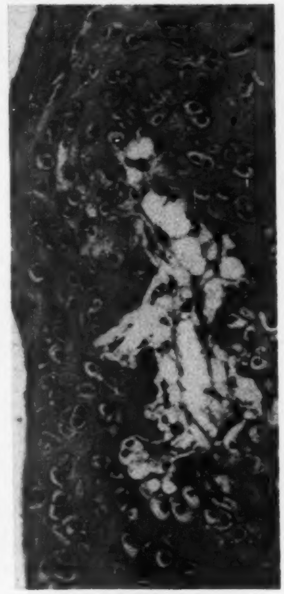

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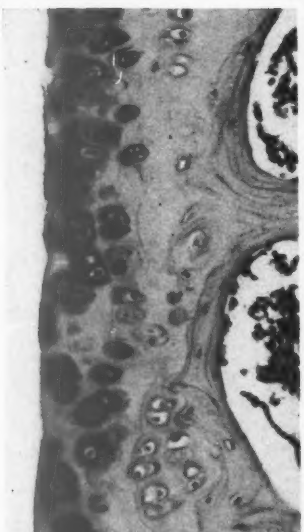

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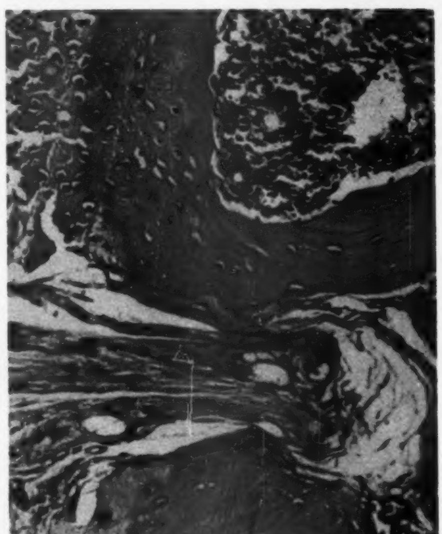

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Skeletal Changes with a High-Fat Diet

PLATE 17

Figures 14 to 18 represent sections through the knee joint of male mice of strain C57 black.

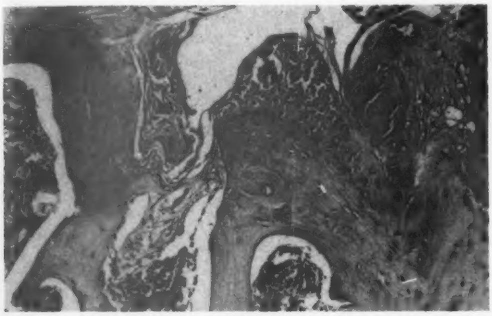
FIG. 14. Four-months-old mouse, fed the high-fat diet for 3 months. Articular surface of the tibia. Loose synovial tissue enters the epiphyseal marrow cavity. At the upper right of the photomicrograph, an area is seen in which the cartilage has undergone fibrillation, vacuolation and fibrinoid change. × 95.

FIG. 15. The area of degenerating cartilage specified in Figure 14 is shown under higher magnification. The fibrinoid material appears somewhat darker than the surrounding matrix. × 225.

FIG. 16. Six-months-old mouse, fed the high-fat diet for 5 months. Articular surface and part of the epiphysis of the tibia. There is an island of fibrous tissue in the epiphyseal bone marrow. × 95.

FIG. 17. Four-months-old mouse, fed the high-fat diet for 3 months. Articular surface of the tibia. A ligament is seen with its insertion. A thick layer of synovial tissue covers the cartilage at the lower left of the photomicrograph. Between the ligament and the synovial tissue is an oblong structure composed of partly hyalinized, partly swollen and degenerated cartilage and two hypertrophic cartilage cells with basophilic capsules.

FIG. 18. The area specified in Figure 17 under higher magnification. At the lower end of the photomicrograph, a small part of the articular cartilage and the underlying bone are seen. The cartilage is covered by closely attached thickened synovial tissue. The ligament is noted at the upper half of the right side of the photograph. The area in the middle shows liquefaction and degenerating cartilage cells. × 225.



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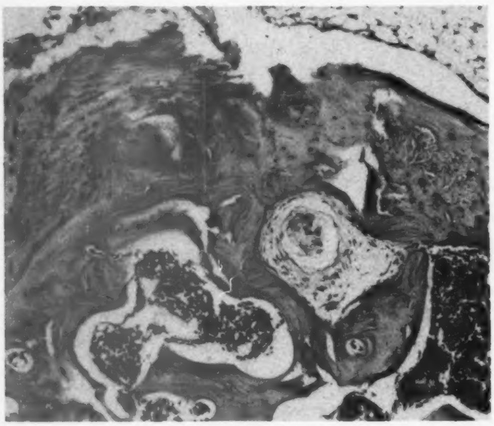

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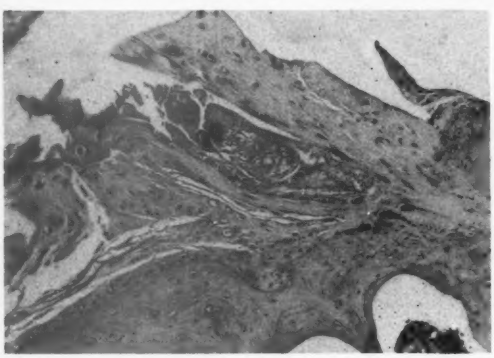

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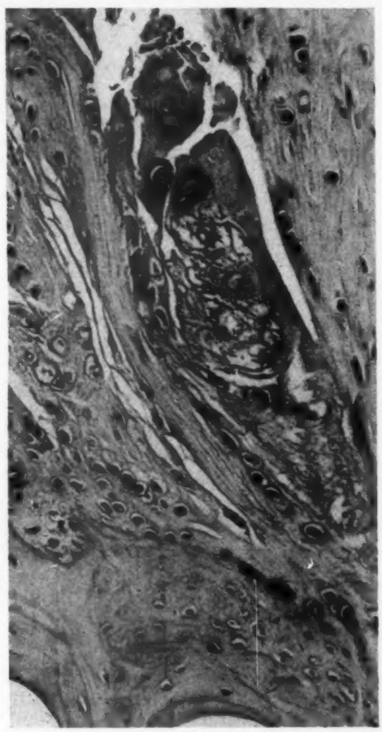

18

INCLUSION ENCEPHALITIS

With a Clinicopathologic Report of Three Cases*

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In 1933 and 1934 Dawson[1,2] reported 2 cases of lethargic encephalitis which he distinguished from the form described by von Economo primarily on the basis of the presence of intranuclear inclusion bodies of type A in cells of the central nervous system. These acidophilic bodies, surrounded by clear halos and marginated basophilic chromatin, have been commonly found in herpes simplex encephalitis and other virus infections. Dawson failed to transmit the disease to rabbits. He was of the opinion that the causative virus in his cases differed from that of herpes simplex, which is easily transmitted to rabbits, and designated the disorder as inclusion encephalitis. Five additional cases of inclusion encephalitis, in which no attempt was made to isolate the virus, have been reported: these are the cases of Akelaitis and Zeldis,[3] Kinney,[4] Brain, Greenfield, and Russell,[5] and Swan.[6] On the other hand, 4 cases of inclusion encephalitis in which the virus of herpes simplex was isolated have been described in the literature: one by Smith, Lennette, and Reames,[7] one by Zarafonetis, Smadel, Adams, and Haymaker,[8] and 2 by Whitman, Wall, and Warren.[9] The last 3 cases have been the subject of a pathologic report by Haymaker.[10]

It is the purpose of this communication to describe 3 additional cases in which the etiology was undetermined and to discuss further the clinical and pathologic aspects of this comparatively unknown disorder.

Report of Cases

Case 1

History. A white male (A.F.I.P. acc. 197268), 17 years of age, was admitted to hospital on February 2, 1947, with a history of increasing psychotic reactions during the preceding 4 months. His personal history was irrelevant. The patient was well until October, 1946, when he began to complain of feelings of depression and tension associated with ideas of reference. He became progressively withdrawn and confused. During January, 1947, he had auditory hallucinations and complained of weakness and soreness of the left side of the body.

Examination. On admission the patient did not appear ill, temperature was normal, and physical (including neurologic) examination was negative. He appeared apathetic, lacked initiative, showed disorientation for time and place,

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impairment of recent and retentive memory, and blocking of speech; he complained of a sensation as of electricity in his body and had waxy flexibility. The initial diagnostic impression was catatonic schizophrenia.

On February 5, 3 days after admission, the patient had an attack of clonic jerking of the limbs, affecting particularly the left side, associated with epistaxis. At this time, neurologic examination revealed a positive Kernig sign, ptosis of the left eyelid, loss of accommodation-convergence of the right eye, weakness of the left side of the face and leg, exaggerated knee jerk with positive Oppenheim and questionable Babinski signs on the right, and a bilaterally positive Hoffmann phenomenon. During the succeeding week the myoclonic jerking continued, but the positive neurologic signs, with the exception of a persistent Hoffmann sign on the right, had disappeared. On February 12 the patient had a grand mal convulsion. From then on he became progressively more stuporous and had to be tube-fed. During early April, myoclonic jerking of the eyes as well as of the limbs was noted, the pupils failed to respond to light, optic fundi were normal, deep tendon reflexes were diminished, abdominal and cremasteric reflexes were absent, and bilateral Babinski signs were elicited. Wasting of muscles became apparent and decubital ulcers began to develop. The temperature now became elevated, varying between 100° and 105° F., associated with cough and expectoration, which were controlled with penicillin. A pill-rolling type of tremor of the left hand was noted on April 28, but disappeared by May 9. On May 17, the eyes deviated upward and to the left, and there were involuntary flexion movements of the right hand, while the signs of pyramidal disorder persisted. There was increasing respiratory difficulty, and on June 27 the temperature rose to 110° F., and death occurred. The duration of the illness was approximately 9 months.

Laboratory studies of the blood revealed 3,500,000 red cells per cmm., hemoglobin of 13.6 gm., leukocytosis varying between 10,300 and 26,400 white cells per cmm., and sedimentation rates ranging between 27 and 35 mm. The chemical constituents of the blood were normal; serologic tests and cultures were negative. Urinalysis also revealed nothing of note. Repeated examinations of the cerebrospinal fluid disclosed clear fluid under normal pressure, containing 5 or 6 lymphocytes per cmm., a trace of globulin, total protein ranging between 18 and 62 mg. per cent, a colloidal gold curve of 5555543210, and a negative Wassermann test. A pneumo-encephalogram was regarded as normal.

Necropsy

With the exception of bronchopneumonia, the significant findings at necropsy were confined to the central nervous system. Grossly, the pia-arachnoid of the brain showed a patchy grayish exudate, engorged blood vessels, and flattening of the convolutions. Over the convex surface the convolutions were abnormally firm, and at the base were softened. On section no other gross abnormalities were noted in the brain or spinal cord.

Microscopic Findings. Hematoxylin and eosin and phloxine and methylene blue stains disclosed numerous intranuclear inclusions in both nerve cells and oligodendroglia. As a rule, these consisted of large, reddish pink, spherical, homogeneous bodies, which replaced the greater part of the nucleus (Figs. 1 and 2). A narrow, slightly beaded

ring of basophilic chromatin usually was separated from the inclusion by a halo; occasionally the peripheral chromatin included a displaced nucleolus. Here and there the inclusions were smaller and granular (Fig. 3), and sometimes were without a halo (Fig. 4). The cytoplasm usually was degenerated, but intracytoplasmic inclusions were not found. The distribution of the inclusions was widespread and uneven, but generally corresponded to areas of maximal pathologic change, namely, in neurons of the cerebral cortex, thalamus, lateral geniculate bodies, and nuclei pontis, and in oligodendroglia of the cerebral and pontile white matter.

The pathologic process consisted of widespread degenerative and inflammatory changes. There was diffuse infiltration of the pia-arachnoid with lymphocytes, plasma cells, and histiocytes. All cortical regions contained numerous perivascular infiltrations of lymphocytes and plasma cells, predominantly around larger vessels. There was variable distortion and rarefaction of the cyto-architecture due to diffuse dropping out of neurons, particularly in lamina III, while remaining nerve cells showed acute swelling, shrinkage, or pallor. Proliferated microglia in the form of rod cells occurred throughout all layers of the cortex, while hyperplastic astrocytes predominated in the deeper layers (Fig. 5). The white matter was even more intensely involved; besides perivascular infiltrations, the picture was characterized by a dense proliferation of clear compound granular corpuscles intermingled with hyperplastic astrocytes (Fig. 5). In the basal ganglia, changes similar to those of the cerebral cortex were most conspicuous in the thalamus and lateral geniculate bodies, while the caudate nucleus, putamen, globus pallidus, hypothalamus, and internal and external capsules near the gray matter were moderately involved. The midbrain contained scattered perivascular infiltrations only in the region of the periaqueductal gray matter. In the pons the changes were concentrated in the basis and brachium, where there were marked disintegration of the tissue and partial replacement by numerous compound granular corpuscles and hyperplastic astrocytes accompanied by perivascular infiltrations (Fig. 6). In the medulla oblongata and cerebellum the process was relatively mild, being limited to scattered infiltrations among the cranial nerve nuclei and in the cerebellar white matter. The spinal cord appeared normal.

A comparison of myelin, glial fiber, and fat preparations revealed a conspicuous disproportion between the demyelination, which was relatively moderate, the gliosis, which was uniformly advanced, and the fat Abbau, which was intense but focal (Figs. 7, 8, and 9). In Spielmeyer and Weil preparations, only scattered areas of the cerebral

white matter and basis pontis showed disintegration of the fibers, while the majority of the sheaths, though swollen, were relatively preserved. By contrast, scarlet red stains disclosed areas of massive fat deposit in compound granular corpuscles of the white matter and in microglia within the gray matter (Fig. 9). In Holzer[11] preparations, the entire cerebral white matter and the basis and brachium pontis were occupied by a dense network of glial fibers in which fibrous astrocytes were scattered; in some areas the gliotic process merged gradually with adjacent gray matter and elsewhere was sharply demarcated from the gray matter (Fig. 8).

Case 2

History. A white girl (A.F.I.P. acc. 204977), 9 years of age, was admitted to hospital on April 25, 1942, with a history of progressive mental and neurologic symptoms during the preceding 1½ years. The family and personal histories were without significance. The patient had been regarded as a normal, intelligent child. In September, 1940, it was noted that she was becoming irritable, neglectful, and was failing in her school work; she began to fall frequently, gradually lost control of her left hand, and exhibited transitory opisthotonus. In December, 1940, she was hospitalized because of continuous vomiting. The possibility of brain tumor was entertained, but she was discharged after a 3-week period of observation. In the spring of 1941, her speech became impaired, she had frequent convulsions, and completely lost the use of her arms and legs. In July, 1941, she began to lose weight and had difficulty in swallowing.

Examination. On admission, physical examination revealed marked emaciation, enlarged tonsils, normal temperature, tachycardia, harsh breath sounds over the lung fields, and blood pressure of 94/70 mm. of Hg. Neurologic examination disclosed clonic spasms, occurring every 6 seconds, which involved the right side of the face and hand and lower extremities, dilated pupils which reacted well to light, optic atrophy, flaccid paralysis of all extremities with diminished tendon reflexes, and no pyramidal or meningeal signs. Mentally, she was entirely unresponsive.

On May 11, 1942, the temperature became elevated, and râles were heard in the chest. By June 9, the temperature dropped to between 100° and 101° F., and there was some return of voluntary movements of the right side. However, on June 30 the patient's condition became worse, and she died on July 2, 1942. The duration of the illness was 21 months.

Urinalyses, blood cultures, agglutination tests, and serologic and spinal fluid studies were negative. Increase in hemal leukocytes from 10,450 to 18,800 per cmm. was the only abnormal laboratory finding. Roentgenograms of the skull disclosed no abnormalities, and a pneumoencephalogram revealed moderate increase in size of the subarachnoid space and the ventricles.

Necropsy

Examination of the organs at necropsy revealed lipoid pneumonia. Grossly, the brain showed moderate thickening of the basal leptomeninges, mild atrophy of the convolutions, diffuse firmness of the cerebrum, brain stem, and spinal cord, and a softened area in the right temporal pole.

Microscopic Findings. As in case 1, numerous intranuclear inclusion bodies of type A were noted in nerve cells and oligodendroglia (Figs. 10 to 13). In this instance, however, there was more variation, smaller and larger bodies with and without halos occurring side by side. Here, too, cytoplasmic degeneration without inclusions was noted. The inclusions varied numerically in different areas, but on the whole corresponded to regions of most intense pathologic change, namely, the cerebral cortex and white matter, pons, medulla oblongata, and spinal cord.

The pia-arachnoid contained scattered infiltrates of lymphocytes and plasma cells. The cerebral cortex was for the most part converted into a spongy state in which there were degenerating neurons, numerous rod cells, large numbers of astrocytes, and scattered perivascular infiltrations with lymphocytes and plasma cells; all cortical layers were involved to about the same degree (Fig. 14). The cerebral white matter also was severely affected, and was occupied predominantly by fibrous astrocytes; compound granular corpuscles were relatively sparse and were confined to perivascular spaces, and only scattered infiltrates of lymphocytes and plasma cells were found. The caudate nucleus, putamen, and thalamus were very spongy and contained numerous astrocytes; inflammatory reaction, however, was minimal. The brain stem and spinal cord, on the other hand, were the seat of severe inflammation and advanced gliosis, the lesions being concentrated in the tegmentum and basis pontis (Fig. 15), the dorsal and olivary portions of the medulla oblongata, and throughout the entire gray matter of the cord (Fig. 16). A prominent feature throughout the brain stem was the presence of numerous glial nodules, consisting of focal proliferation of microglia, either free in the tissue or in the process of phagocytosing degenerated neurons. The cerebellum showed diffuse degeneration of the Purkinje layer and dentate nucleus. The changes in the white matter of the cerebellum and spinal cord were relatively mild.

In this case there was a closer correspondence between degree of demyelination and gliosis. Thus, large areas of the cerebral white matter and fibrae pontis, exclusive of the pyramidal tracts, showed advanced demyelination which could be correlated with intense gliosis in Holzer-stained sections. However, even in this case, some areas in which myelin sheaths were preserved displayed beginning gliosis.

Case 3

History. L. J. B., a white girl (L.P.C. 393), aged 10 years, was admitted to hospital on November 29, 1939, with a history of spells of unconsciousness, failing

vision, and signs of intellectual impairment, all of 6 weeks' duration. The family and personal histories were without significance. The patient was regarded as of better than average intelligence prior to onset of her illness. About 6 weeks before admission, she became subject to spells characterized by sudden staring expression, stiffening of muscles, and falling to the ground; these lasted several minutes. At first the seizures occurred about once a week in a series of four to five attacks in the course of 1 hour; later they became more frequent. At the same time her school work began to decline and examination by an optometrist disclosed impaired vision of the right eye.

Examination. On admission, the patient was moderately obese, temperature was normal, and general physical examination was negative. Ophthalmologic examination revealed vision of 18/200 on the right and 20/30 on the left, with mild optic atrophy on the right and bilateral degeneration of the macula, with diffuse hyperpigmentation of the rest of the retinae. The neurologic examination was otherwise negative. Mentally, her responses were slow and a Stanford-Binet test indicated deterioration, the I.Q. being 73. Roentgenograms of the skull were negative and laboratory studies were normal, but the cerebrospinal fluid was not examined. A diagnosis of cerebromacular degeneration was made and the patient was discharged from the hospital.

Re-examination in March, 1940, revealed further intellectual deterioration, the I.Q. now being 66. At this time there was ankle clonus with an equivocal Babinski sign on the right side. Subsequently, her speech gradually began to deteriorate until complete mutism was reached. There were momentary spells of unconsciousness initiated by turning movements to the right. An episode of complete paralysis associated with incontinence and followed by remission after 1 year was reported by the family. Re-examination in April, 1943, disclosed such advanced mental deterioration that the patient was no longer accessible to psychologic testing. On February 15, 1946, the patient was admitted to a State hospital.* At this time the principal features of her disease consisted of complete dementia, blindness with the same changes in the fundi as previously noted, spastic weakness of all extremities with exaggerated tendon and absent abnormal reflexes, and a positive Babinski sign on the right. Jacksonian-like attacks confined to the right lower extremity occurred about three times a week. In spite of the advanced stage of her illness, there were periods of partial remission of the paralysis followed by relapse. Death occurred on October 3, 1946. The duration of the illness was approximately 7 years.

Necropsy

Examination by necropsy confirmed a terminal bronchopneumonia. The brain weighed 1020 gm. There was moderate atrophy of the cerebral convolutions. The pia-arachnoid was diffusely thickened while the blood vessels appeared normal. Coronal sections revealed marked reduction of the cerebral white matter and of the corpus callosum, with bilaterally symmetric dilatation of the lateral ventricles. In the frontal lobes, the central parts of the white substance were gray and of gelatinous consistency, while in the other lobes the white matter was usually white and firm. Through the cerebrum, the gray matter, though pale, was demarcated sharply from the white substance.

In large Weigert preparations there was advanced demyelination of

* The staff of Napa State Hospital, California, cooperated in furnishing this material.

the cerebral white matter of all regions, with the exception of the pre-rolandic and post-rolandic areas. The demyelination was present chiefly in the central parts of the white substance, whereas the gyral white matter, including the U fibers, was relatively well preserved. The myelo-architecture of the cortex was generally destroyed. The corpus callosum was severely demyelinated, but the pyramidal fibers and the optic pathways were relatively spared. There was also severe demyelination of the dorsomedial and anterior nuclei of the thalamus, while the remaining basal ganglia, brain stem, and cerebellum were minimally involved.

Microscopic Findings. Numerous intranuclear inclusion bodies were demonstrated, particularly by means of the phloxine and methylene blue method. In this case, however, the inclusions differed somewhat from those in the previous 2 cases in that they stained lavender rather than reddish pink and were surrounded immediately by a basophilic ring of chromatin, usually without halo formation (Fig. 17). Moreover, they were restricted virtually to the nerve cells of the cerebral cortex, there being only a few in oligodendroglia and then they filled the nucleus completely.

In Nissl preparations the pia-arachnoid was infiltrated diffusely with lymphocytes, plasma cells, and histiocytes. The cerebral cortex of all regions contained numerous perivascular infiltrates; around larger vessels the cells were predominantly lymphocytes, and around capillaries, mainly plasma cells (Fig. 18). Countless microglial elements, both as rod cells and as glial rosettes lying free in the tissue, were found in all layers; also occasionally neuronophagia was observed (Fig. 19). The neuroglial reaction was moderate and consisted of proliferated astrocytes, particularly in the deep layers. The cytoarchitecture was distorted or obscured by inflammatory cells and proliferated glia. There was a diffuse dropping out of neurons and many of those that remained were swollen, shrunken, and ghost-like. The changes were most severe in laminae III and V. In Nissl preparations the severely demyelinated white matter showed diffuse increase in small glial nuclei, more so in subcortical than in central parts, and fairly numerous perivascular infiltrations with plasma cells and lymphocytes or with pigment-laden gitter cells (Fig. 20). In the basal ganglia and brain stem the changes in the gray masses were as severe as in the cerebral cortex, but the white matter was only slightly involved. Numerous perivascular infiltrations and glial nodules were present in the thalamus (Fig. 21), geniculate bodies, pretectal area, substantia nigra (Fig. 22), and pontile and dentate nuclei. The caudate nucleus, puta-

men, and tectal and tegmental regions of the brain stem were moderately involved, and only slight changes were noted in the globus pallidus, hypothalamus, red nucleus, and inferior olivary nucleus. The cerebellum showed patchy degeneration of the Purkinje layer. The spinal cord was not available for study.

A comparison of myelin and Holzer preparations revealed close correspondence in degree of intensity of demyelination and gliosis (Figs. 23 and 24). However, in the Holzer preparations the sharply delineated gliosis of the cerebral white matter involved the U fibers as well, which was in contrast to their relative integrity in myelin preparations. In sections stained with scarlet red only a scant amount of fat was noted in gitter cells in the perivascular spaces. With the Turnbull blue method, there was no evidence of iron deposit in glia or blood vessels.

An unusual feature of this case was the presence of intracellular fibrillary alterations in numerous neurons of the cerebral cortex, thalamus, midbrain, and rest of the brain stem (Figs. 25 to 27). Fibrillae were greatly thickened and tortuous, often basket-shaped, and occupied the periphery of the cytoplasm. They stained best with silver methods, and they resembled the basket cells of Alzheimer's disease, but were unassociated with senile plaques. In nerve cells in the region of the aqueduct, large well circumscribed amorphous masses containing traces of tangled degenerating fibrillae were found in the cytoplasm (Figs. 28 and 29). Appearances indicated that they progressively enlarged and replaced the nucleus, probably representing an end stage of the neurofibrillary alteration.

Comment

Clinical Features

The 3 cases reported here illustrate a hitherto unobserved chronic type of inclusion encephalitis. They are arranged in the text in order of increasing chronicity. The age of onset of the illness was 17, 9, and 10 years, respectively, which corresponds to previous observations that this disease is virtually limited to the first 2 decades of life. The onset was insidious, the course slowly progressive, and the duration 9 months, 21 months, and 7 years, respectively. In this respect our cases differ from those reported in the literature, in all of which the disease ran an acute or subacute course, with the exception of one of Dawson's cases[1] in which it was of 16 months' duration and was marked by a long period of remission. The clinical picture in the present series differed from the syndrome noted by other authors and thus presented a diag-

nostic problem. Mental deterioration made its appearance early and was striking, varying from the schizophrenia-like personality disorder in case 1 to the progressive deterioration of intellectual and speech faculties in cases 2 and 3. Convulsions, either petit or grand mal, generalized or jacksonian, occurred during different phases of the illness. Optic atrophy was noted in case 2, and macular degeneration with progressive blindness in case 3. Other features were prominent motor disturbances in the form of pyramidal, extrapyramidal, or lower motor neuron signs, and myoclonic spasms; meningeal and ocular signs were fleeting in character. In none of the cases was there an elevation of temperature until terminally, when it was associated with the development of pneumonia. Herpes labialis or other forms of dermatitis were not observed.

Laboratory studies were noncontributory and the cerebrospinal fluid, as reported in other cases in the literature, was negative, with the exception of a first-zone curve in case 1, a feature also observed in the case reported by Akelaitis and Zeldis.[3]

It should be emphasized that this syndrome, characterized by a combination of progressive mental deterioration and variable neurologic manifestations referable to widespread involvement of the central nervous system, is unique among the various forms of virus encephalitis.

Patho-anatomic Features

The specific pathologic characteristic common to all of these cases was the presence of intranuclear inclusions. In cases 1 and 2, the majority of the inclusions displayed the classic features of the type A form. However, some of the inclusions in the first 2 cases and virtually all of those in case 3 differed from the typical A form in that they were less acidophilic and lacked characteristic halos. This latter feature may have its explanation in the observation of Cowdry[12] that the more chronic the virus infection the greater the tendency for the halos to disappear because of nuclear shrinkage. The inclusions were most numerous in neurons, but were also noted in oligodendroglia, particularly in cases 1 and 2, their distribution generally corresponding to areas of maximal histologic change and, therefore, directly associated with cellular damage caused by the hypothetical virus. No cytoplasmic inclusions were demonstrated. While the presence of intranuclear inclusions suggested probable virus infection, as postulated by Cowdry, in the absence of animal inoculation studies the etiologic agent could not be determined. In differential diagnosis, herpes simplex encepha-

litis could not be ruled out on morphologic grounds alone, although, as a rule, in herpes simplex encephalitis the inclusions are considered more pleomorphic.

The chronicity of the cases reported here is not considered a valid criterion for separating them etiologically from the acute cases in which herpes virus has been isolated. Experimentally, Good and Campbell[13] have found in rabbits that herpes virus may produce either acute or chronic smouldering encephalomyelitis. The factors which determine the variable course of the infection are not known.

Patho-anatomically, the type of change in our cases was similar to that reported by previous authors, the differences depending on the greater chronicity of our cases. Thus, in acute forms, necrosis and hemorrhage in regions of inflammatory reaction have been emphasized. In our cases, on the other hand, gliosis and demyelination were conspicuous in spite of the still active inflammatory process. In this respect a sequence in the evolution of the changes was demonstrated in the 3 cases. Thus, while demyelination was comparatively mild and gliosis was severe in case 1 which was of relatively shorter duration, there was almost equally severe demyelination and gliosis in cases 2 and 3, of longer duration. The disease affected the entire central nervous system, gray and white matter equally, from the cerebral cortex to the spinal cord, in varying combinations. In distinguishing it from other types of encephalitis, it would seem that this disease combined the features of those forms predominantly affecting gray matter on the one hand, and those involving primary white matter on the other. Thus, in the gray matter the reaction was principally mesodermal, consisting of meningeal and perivascular infiltrations of lymphocytes and plasma cells, glial nodules and rod cells. In the cerebral cortex the process was not unlike that of general paresis, while in the brain stem it resembled various epidemic forms of encephalitis. The changes in the white matter were primarily ectodermal, characterized by gliosis and demyelination, much as in certain inflammatory diseases.

In these patho-anatomic changes, our cases bore a remarkable resemblance to a disorder reported in recent years by Van Bogaert[14] under the designation subacute sclerosing leuko-encephalitis. It is noteworthy that there were also striking similarities in respect to age incidence, symptomatology, and clinical course. There was, however, no mention of the presence of intranuclear inclusions in the cases reported by Van Bogaert, and therefore it is impossible to state whether we are dealing with the same disorder. Van Bogaert emphasized the disparity between the severe gliosis and the relatively normal myelin

picture in his cases, all of which ran a subacute course. On the basis of this he distinguished the disorder from the primary demyelinating diseases and proposed the term leuko-encephalitis. While we are in agreement with Van Bogaert that all of these cases represent primary inflammatory disorders and are to be distinguished from the demyelinating diseases, the term leuko-encephalitis, in our opinion, is confusing. It tends to overemphasize the affection of the white matter in a condition in which gray and white substance are equally involved.

An interesting feature of case 3 was the widespread neurofibrillary alteration which resembled the basket cells of Alzheimer's disease, although the senile plaques were lacking. Such a finding in a young person is of particular significance. It is to be noted that case 3 represents the most chronic form of inclusion encephalitis hitherto observed. It recalls Hallervorden's[15] observation of Alzheimer fiber changes in cases of chronic epidemic encephalitis. Such observations would tend to re-emphasize the conclusion that the phenomenon of Alzheimer fiber change is not a specific reaction related to senile involution, but occurs on the basis of different etiologic factors and has wider pathogenic significance.

Summary

The three cases of inclusion encephalitis which are reported illustrate unusually chronic varieties of a disorder first described by Dawson.[1,2]

Progressive mental deterioration associated with variable neurologic signs referable to widespread involvement of the central nervous system characterize the disorder.

The patho-anatomic substratum is a diffuse encephalitis involving equally the gray and the white matter with intranuclear inclusions of type A, which are morphologically indistinguishable from those of herpes simplex encephalitis. Alzheimer's neurofibrillary alterations were observed in the most chronic case of the series.

The disorder is compared with that reported by Van Bogaert as subacute sclerosing leuko-encephalitis.

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DESCRIPTION OF PLATES

Plate 18

All sections used for this plate were from case 1 and were stained by hematoxylin and eosin.

Fig. 1. Intranuclear inclusion in ganglion cell of cerebral cortex. A halo separates the inclusion from the beaded basichromatin attached to the nuclear membrane. × 1200.

Fig. 2. Oligodendroglial cell of cerebral cortex containing intranuclear inclusion. × 1300.

Fig. 3. Medium-sized intranuclear inclusion in ganglion cell of cerebral cortex. Clumps of nuclear chromatin still are present. The globular structure to the left is considered to be a partially extruded nucleolus. × 1200.

Fig. 4. Intranuclear inclusions completely filling two ganglion cells of the basis pontis. As in the other cells, the cytoplasm has undergone disintegration × 1200.

Fig. 5. Cortical-subcortical junctional area. The cortex shows a reduction in ganglion cells and there are many proliferating astrocytes, especially in the white matter. × 150. (The arrow points toward the meninges.)

Fig. 6. Section of the basis pontis which shows inflammatory exudate around a vessel, marked reduction in ganglion cells, and proliferation of astrocytes. × 100.

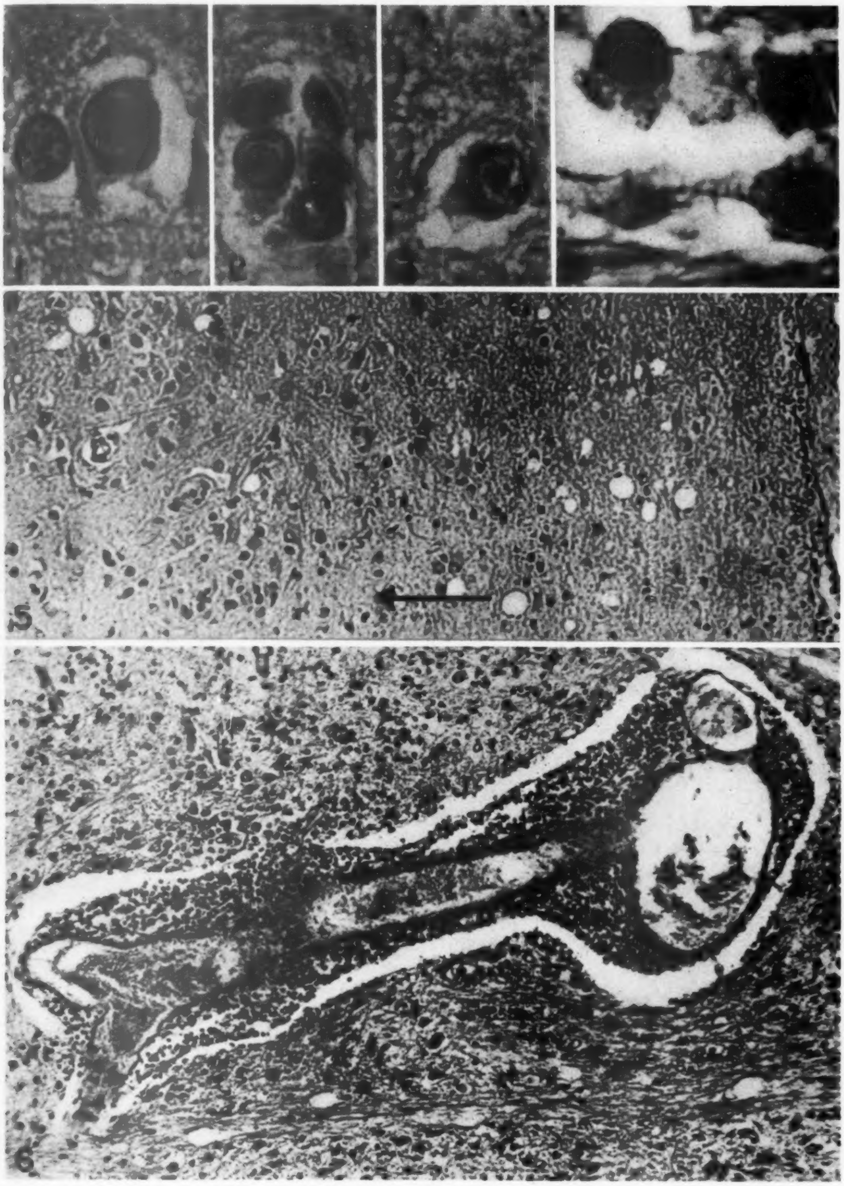

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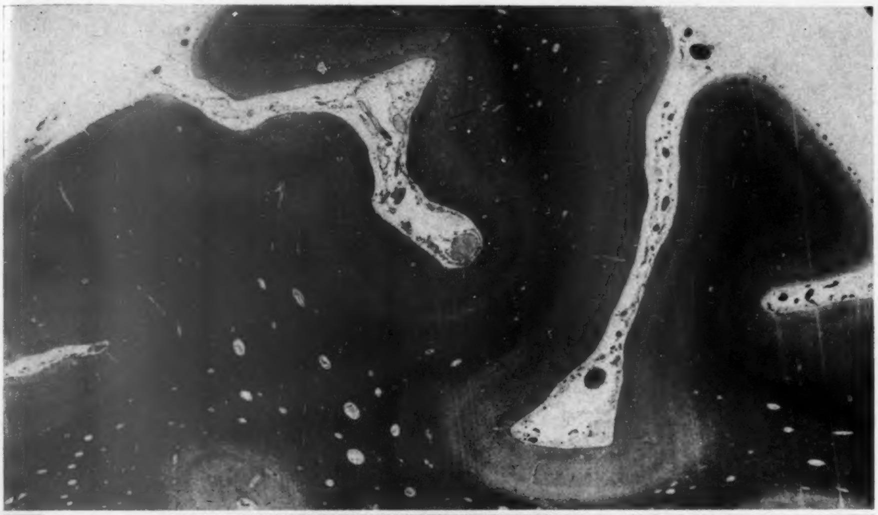
PLATE 19

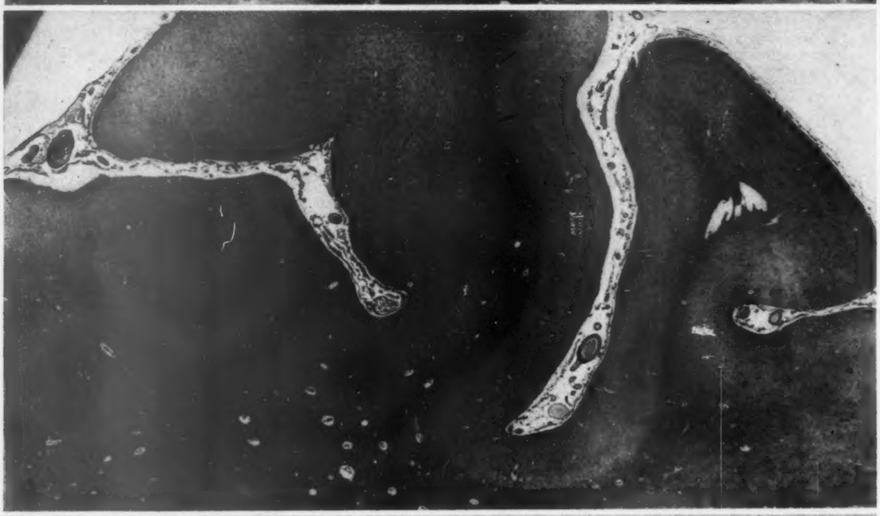
All sections used for this plate were from case 1.

FIG. 7. Section of cerebrum showing scanty myelin rarefaction. Weil myelin stain. × 6.

FIG. 8. Section corresponding to that of Figure 7 but stained by the Holzer method.[11] There is moderately advanced gliosis which encroaches on the cortex. × 6.

FIG. 9. Section from cerebral cortex showing myriad fat-containing compound granular corpuscles, especially in the subcortical white matter. Scarlet red stain. × 100. (The arrow points toward the meninges.)





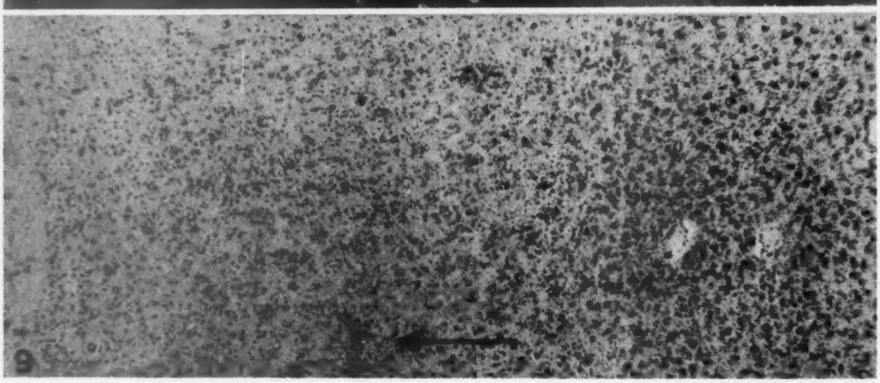

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Plate 20

All sections used for this plate were from case 2.

Fig. 10. Intranuclear inclusion in oligodendroglial cell of cerebral cortex. Hematoxylin and eosin stain. × 1300.

Fig. 11. Intranuclear inclusion in ganglion cell of pons. The nucleolus is enlarged and is somewhat eccentric. Hematoxylin and eosin stain. × 1200.

Fig. 12. Intranuclear inclusion in ganglion cell of pons. Nucleolus is lodged against the nuclear membrane as in Figure 11. The inclusion is surrounded by a narrow halo. Hematoxylin and eosin stain. × 1300.

Fig. 13. Intranuclear inclusion in ganglion cell of cerebral cortex. The inclusion completely fills the nucleus. Hematoxylin and eosin stain. × 1300.

Fig. 14. Cerebral cortex showing rarefaction, marked dropping out of ganglion cells, and proliferation of glial elements. One vessel is surrounded by a few lymphocytes. Thionin stain. × 48. (The arrow points toward the meninges.)

Fig. 15. Basis pontis, in which many ganglion cells have dropped out and in which there is hyperplasia of varied glial elements, especially astrocytes. A sparse perivascular accumulation of lymphocytes is to be noted. Thionin stain. × 65.

Fig. 16. The spinal cord showing gray and white matter. Large perivascular collections of lymphocytes are present at the base of the posterior horn of gray matter. There is scattered proliferation of glial cells both in the posterior horn and the anterior horn. Thionin stain. × 62. (The arrow points to the anterior horn.)

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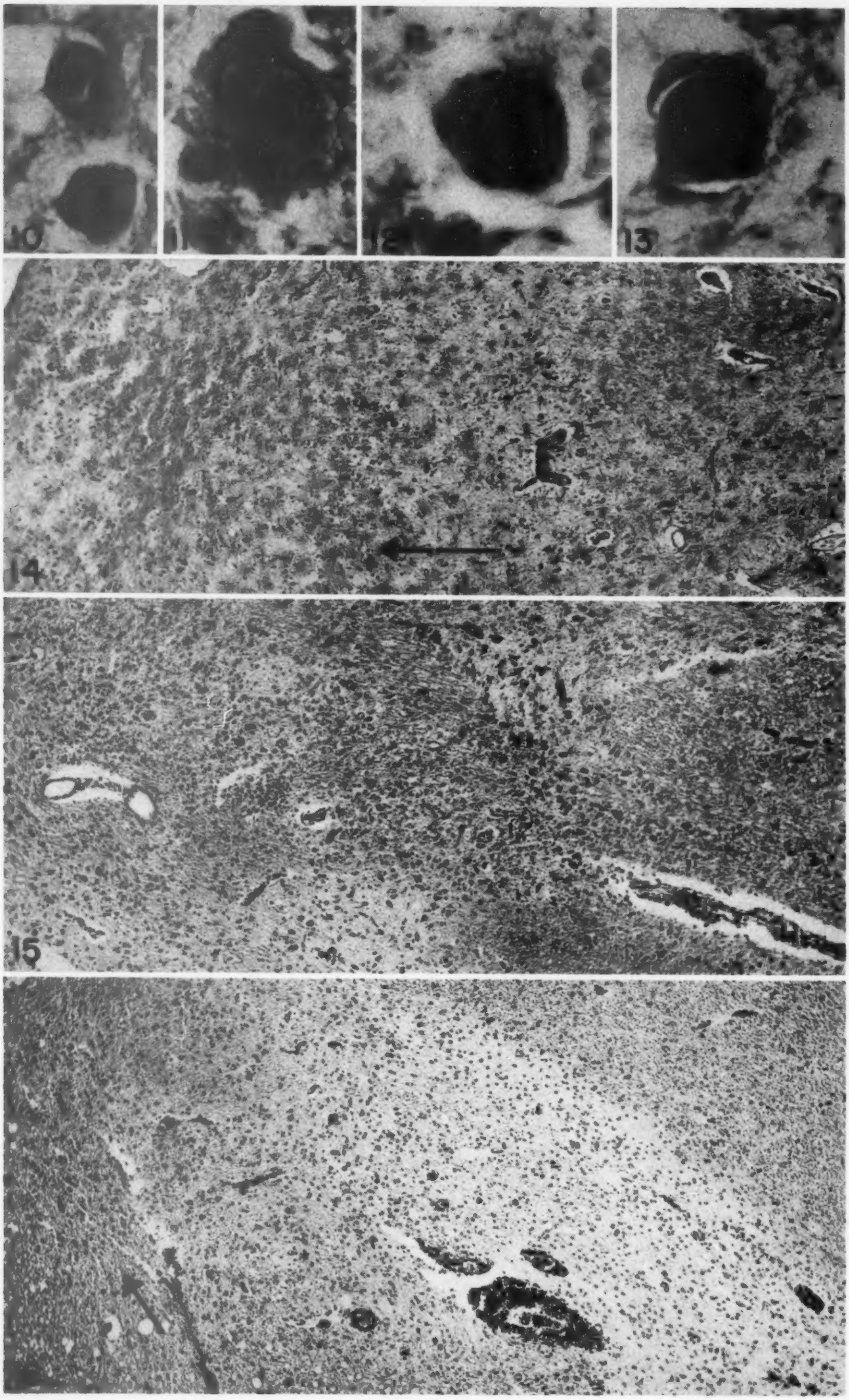
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PLATE 21

All sections used for this plate were from case 3.

FIG. 17. Intranuclear inclusion body in ganglion cell of cerebral cortex. Hematoxylin and eosin stain. × 1300.

FIG. 18. Cortex of occipital lobe showing some reduction in number of ganglion cells and proliferation of glia, some in the form of neuronophagic nodules. There is increase of glia also in the subcortical white matter. Thionin stain. × 62. (The arrow points toward the meninges.)

FIG. 19. Cerebral cortex showing very numerous microglial elements and scattered, sparse perivascular lymphocytes. In the left lower corner of the photograph is a neuronophagic nodule. Thionin stain. × 120.

FIG. 20. Cortical-subcortical junctional area showing increase in glia and perivascular mononuclear cells. Thionin stain. × 90.

FIG. 21. Pulvinar of thalamus showing intense perivascular exudate, loss of ganglion cells, and proliferated glia, some in the form of neuronophagic nodules. Thionin stain. × 62.

FIG. 22. Substantia nigra showing changes similar to those in Figure 21. Thionin stain. × 80.

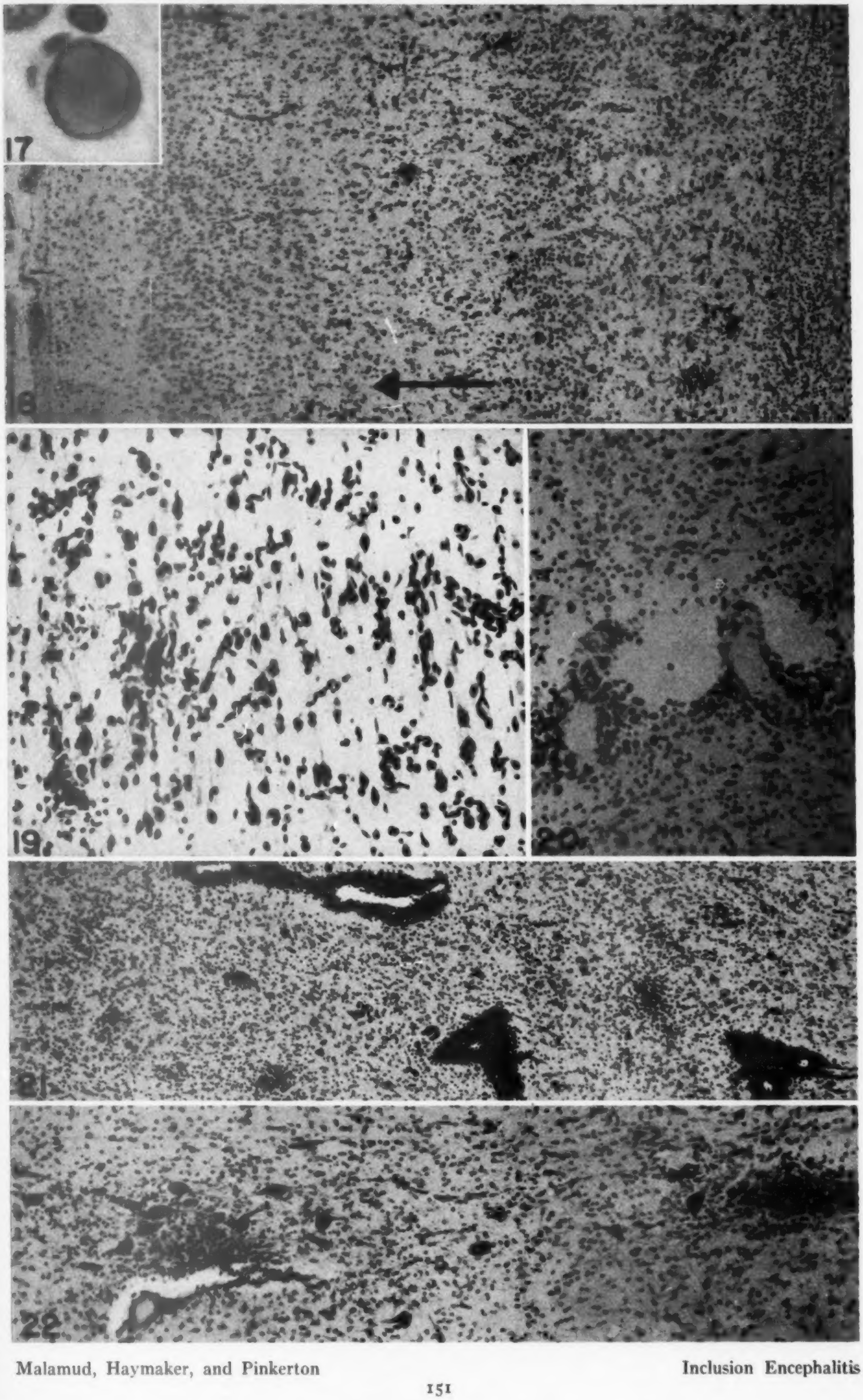
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PLATE 22

All sections used for this plate were from case 3.

FIG. 23. Section of cerebrum showing severe demyelination of the white matter with preservation of U fibers. Weil myelin stain. × 8.

FIG. 24. Section corresponding to that in Figure 23 but stained by the Holzer method. The gliosis is of about the same degree as the demyelination shown in Figure 23. × 8.

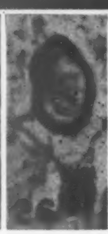
FIG. 25. Neuron of substantia nigra showing Alzheimer glial fiber change. Much of the cell body has undergone disintegration. Hematoxylin and eosin stain. × 400.

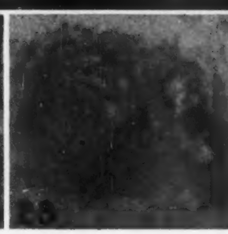
FIG. 26. Ganglion cell of cerebral cortex showing Alzheimer glial fiber change around the periphery of the cell. Von Braunmühl stain.[16] × 400.

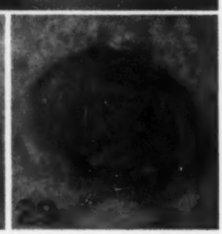
FIG. 27. Another example of Alzheimer glial fiber change in a ganglion cell of the cerebral cortex. Von Braunmühl stain. × 800.

FIG. 28. Ganglion cell in periaqueductal region of upper pons showing accumulation of finely fibrillary material in the cytoplasm. The nucleus is eccentric. Alzheimer glial fiber stain. × 900.

FIG. 29. Ganglion cell in similar location as in Figure 28 in which the cytoplasm and nucleus are completely replaced by a fibrillary body. The crescent-shaped body to the right is believed to be the remainder of the nucleolus. Alzheimer glial fiber stain. × 900.







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DISSEMINATED ARTERIOLAR AND CAPILLARY PLATELET THROMBOSIS

A Morphologic Study of Its Histogenesis*

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The report of 4 cases of disseminated arteriolar and capillary platelet thrombosis by Baehr, Klemperer, and Schifrin[1] in 1936 established the disease as a clinical as well as a pathologic entity, although Moschcowitz[2] had reported the first instance in 1925. To date a total of 16 cases have been described in the literature. However, the increasing frequency with which the reports have appeared suggests that the condition is not so rare as that figure would indicate. Four of the cases were published in two excellent reviews[3,4] in 1947; the most recent case was that of Muirhead, Crass, and Hill,[5] in 1948.

The significant features of the syndrome, as it has been described, are insidious onset of vague non-localizing symptoms, fever, purpura, anemia, and a rapidly progressive fatal course in which severe non-localizing mental and neurologic signs are prominent. Thrombocytopenia, moderate and variable leukocytosis, reticulocytosis, and immature red cells characterize the blood picture. Often the differential count exhibits a distinct "leftward shift" of the myeloid cells, which impressed one group of investigators sufficiently to cause them to designate it a leukemoid reaction.[4] Tests of the clotting mechanism demonstrate increased bleeding time and defective clot retraction; the coagulation time is usually normal. Osmotic fragility of the red cells is unaltered; there is increased capillary fragility; and icterus, generally of a mild degree, occurred in 8 of the 16 reported cases. Contrary to an earlier impression,[6] males, both white and colored, are affected as well as females.[3] The ages in the published cases range from 9 to 66 years. The duration of the terminal illness varied from 1 week to 2 months.

The gross features at autopsy, save for widespread petechiae and ecchymoses, are not distinctive. Histologically, widely disseminated occluding "thrombi" in arterioles and capillaries have been described in almost all organs of the body. It is the consensus of the reporting authors that agglutinated platelets provide the bulk of the occluding

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material. Associated with the platelet plug and considered a consequence of it, there is conspicuous proliferation of the endothelium. The variability of the endothelial reaction, from absence to marked proliferation encompassing and invading the thrombus, has led to the conclusion that the process was intermittent and that occlusions occurred in showers or crops.[6,7] It is the variability of the endothelial reaction which also gives support to the prevalent view that there is not a primary vascular lesion to account for the agglutination and deposition of platelets. Foci of ischemic parenchymal necrosis are a consequence of the circulatory impairment, but necrosis is disproportionately slight considering the widespread distribution of the thromboses. The removal from the circulation of the large number of platelets required to form the myriad thrombi is regarded as the cause of the severe thrombocytopenia,[2] a mechanism altogether different from that proposed for idiopathic thrombocytopenic purpura.[8]

Materials and Methods

In view of the current interest in the problem, it was considered of value to review the 5 (unreported) cases of this disease on file at the Armed Forces Institute of Pathology.* The clinical features were summarized and are presented in Table I together with prominent associated but not obviously related, clinical and pathologic data. At autopsy, widespread purpura was present in all; appreciable splenomegaly was observed in cases 2, 3, and 4. In other respects the gross autopsy findings were nondescript. The tissues from each case were studied microscopically using the following stains and methods: hematoxylin and eosin, Masson's trichrome, Giemsa, phosphotungstic acid hematoxylin, Wilder's reticulum, Weigert's elastica, and Schiff.[35] On a few representative sections the cresyl violet stain for amyloid was employed, and frozen sections were stained for fat with sudan III. Slides of 4 previously reported cases[4,6,7] obtained through the courtesy of Drs. Paul Klemperer, Otto Saphir, and Monroe Schlesinger were available for purposes of comparison.

Pathologic Observations

Platelet thrombi occluding the capillaries and arterioles were the most striking microscopic feature in all tissues examined. These included the heart muscle, pancreas, adrenal, kidney, pituitary gland, brain, intestinal tract, diaphragm, lung, trachea, thyroid, liver, spleen,

* Two cases (A.I.P. acc. 100486 and 110486) were made available through the courtesy of Dr. Nathan B. Friedman while he was a member of the staff of the Armed Forces Institute of Pathology. Dr. Friedman plans to publish a separate study on this material.

TABLE I

Clinical Features of Five Cases of Disseminated Capillary and Arteriolar Platelet Thrombosis

Case no.	A.I.P. acc.	Race	Age	Sex	Prodromal symptoms	Hemorrhagic manifestation	Pallor	Jaundice	Hepatomegaly	Splenomeglay	Mental and neurologic manifestations	Fever, 101°-104° F.	Gastro-intestinal	Hematuria	Severe anemia	Leukemoid reaction	Thrombopenia	Duration	Miscellaneous clinical and pathologic data
1	100486	W	25	F	Fatigue, pallor, and weakness for 10 months; bruising for 1 week	+	+	+	–	–	Headache, delirium, convulsions, coma	+	Melena	+	+	+	+	14 days	Spontaneous abortion at 2½ months
2	110486	N	23	M	Night sweats, 4 months; cough, 1 month; fever, 10 days	–	NR	+	–	+	Coma, spasticity, Cheyne-Stokes respiration, convulsions	+	–	+	+	o	o	30 days	Tuberculous enteritis, mesenteric adenitis, splenitis, and hepatitis; positive skin tuberculin reaction
3*	121863	W	21	M	Weakness, headache, pallor, and malaise for 6 months	+	+	+	–	–	Coma	+	Slight rectal bleeding	–	+	+	+	4 days	Numerous transfusions, hemosiderosis of spleen and liver, subacute (nephrotic) glomerulonephritis (5½ years); terminal septicemia from abscess of buttock
4*	121864	W	44	M	Headaches, abdominal pain, otalgia, dizziness, and deafness, for 3 weeks; aphasia for 1 day	+	+	+	–	+	Headache, dizziness, deafness, aphasia, convulsions, and coma	+	–	+	+	–	+	13 days	2 year occupational history of lead exposure
5	193721	N	53	M	Dyspnea, substernal pain, and urinary frequency for 9 weeks	–	NR	–	–	–	Sudden confusion and disorientation; questionable weakness of both upper and the right lower extremities	o	–	–	+	o	o	4 days	Sicklemia

* Contributed from the Department of Pathology, Columbia University College of Physicians and Surgeons. Courtesy of Dr. Smith.
+ = Present. – = Absent. O = No information. NR = Not reported.

and bone marrow (vertebra and rib). While the structure of the thrombi could not be ascertained when they were compact and formed an amorphous or granular mass, the individual platelets could be seen in more loosely agglutinated portions. Plugs of platelets obtained by centrifuging normal human blood presented similar morphologic features and staining reactions, when they were fixed, embedded, and sectioned in the same fashion. In identifying platelets, and particularly in differentiating a platelet thrombus from a mass of fused red cells, the Schiff staining reaction was found to be more helpful than other stains. Platelets assumed a pink hue which distinguished them readily from the erythrocytes which were yellow. In case 5, complicated by sicklemia, the Schiff reaction permitted, with considerable assurance, the differentiation of platelet masses from clumps of sickled red cells.

None of the venules appeared to be involved in the process. Often, however, the presence of a thrombus in an arteriole so distended it and so attenuated its wall that the usual features differentiating it from a venous channel were lost. Accurate identification could then be made only from the structure of contiguous sections of the vessel at a distance from the occluding (and distending) lesion, or by the position of the vessel in relation to other histologic structures such as renal glomeruli or splenic follicles. In agreement with all previous observations, considerable variation was noted in the degree of endothelial reaction to the platelet thrombus. Frequent absence of this reaction, it will be recalled, led many to exclude a primary vascular lesion. In other instances there was striking proliferation of endothelial cells. While the majority of the lesions at autopsy were relatively recent, there was usually a smaller number of organizing and organized occlusions, which led to the inference, previously arrived at by others, that the platelet-thrombosing process was episodic and that lesions occurred in crops.

Microscopic foci of ischemic necrosis were often found, but seemed relatively insignificant in view of the widespread distribution of the occluding lesions. Bernheim[6] suggested, in this regard, that incomplete occlusion and lack of involvement of venous channels permitted sufficient collateral circulation to minimize parenchymatous damage. Allowing for the reaction of necrotic tissue, the inflammatory reaction to the thrombi was remarkably bland. The entrapment of myriad platelets in the multiple thrombi seems a reasonable explanation of the thrombopenia observed during life, as reported by Baehr, Klemperer, and Schifrin.[1]

Megakarocytes were increased in number in the specimens of bone

marrow available for examination; they were frequently noted also in the capillaries of the lung, an observation previously made by Singer, Bornstein, and Wile.[4] There was no clear evidence of arrest of megakaryocytic maturation and differentiation as described by Dameshek and Miller[8] in cases of hypersplenism with thrombopenia.

Two additional observations seemed to warrant closer scrutiny since they concerned the pathogenesis of the lesion. The paucity of platelet thrombi in the lung capillaries was striking, not only in the cases here reported but also in four others examined by the author. There was a similar exemption of the liver sinusoids, although involvement of the arterioles in the portal spaces was frequent. This seemed to be the reverse of what would be anticipated if the lesions were secondary to spontaneous agglutination in the circulating blood. On the other hand, when the section fortuitously included an appreciable length of an involved vessel, propagated platelet thrombi covered by a sleeve of hyperplastic endothelium were often observed. The proliferating, plump, investing endothelial cells contrasted sharply with the flat, tenuous lining of the occluded vessel and indicated an origin from a portion of the vessel not always in the plane of the tissue section. Careful search did indeed reveal focal lesions of arterioles and capillaries upon which platelet thrombi formed and from whence they grew along the length of the vessel. These unique vascular changes have not been described before, an oversight probably arising from their focal nature, the rapidity of their evolution, and the prompt response of the clotting mechanism to endothelial damage. The great majority of the thrombi in sections from autopsy material are seen in transection at a distance from the initiating lesion.

The focal lesions here termed "prethrombotic" were quite rare. Among the cases available for review they could be demonstrated in the myocardium, pancreas, bone marrow, hilar lymph nodes, liver, and skin. The vessels involved were chiefly arterioles but included an occasional capillary. The lesion consisted of a segmental accumulation of hyaline material beneath the endothelium of a capillary and between the endothelium and musculature of an arteriole. The Schiff periodic staining reaction proved of great value in its demonstration, since the hyaline areas assumed a bright red hue which contrasted vividly with the yellow color of the remaining structures (Figs. 1, 2, and 3). In focal areas swelling of this homogeneous substance was noted, so that it bulged both into the vessel lumen, carrying with it the overlying unaltered endothelium, and externally, to produce a defect in the vessel wall. It is possible that the lesions regarded as extruded thrombi by two pre-

vious observers[3,9] were of this type. With the reticulum stain, however, the splitting of the components of the vessel wall by the hyaline mass and the integrity of the overlying subendothelial argyrophilic membrane demonstrated this to be a lesion *sui generis* rather than a herniated thrombus (Figs. 4 and 5). Cresyl violet stains for amyloid and sudan III stains of frozen sections for fat gave negative results.

Although the nature of the change cannot be established by ordinary histologic technics, the swelling to which the hyalin is subject can best be explained by the imbibition of fluid and possibly other substances from the circulating blood. Edema of the vessel wall adjacent to the hyaline nodule in Figure 6 may be an indication of such a transfer of fluid. At any rate, the swelling evidently progressed until there was a break in the overlying endothelium, whereupon platelets accumulated rapidly to cover the defect (Fig. 7); thus a bimorphic thrombus was formed with components contributed both from the vessel wall and from the blood (Fig. 8). The great rarity of such bimorphic thrombi and the frequency of the monomorphic variety suggest that the hyaline substance from the vessel wall disintegrates or dissolves rather promptly on exposure to the circulating blood. It seems reasonable to assume that the sequence just described occurs within a short interval, else the well known capacity of the endothelium to proliferate promptly would prevent its rupture. It is to be noted that significant proliferative endothelial reaction was absent in this early phase, but developed subsequently from the intact marginal endothelium to extend over the thrombus. At a later stage it was often noted that the vessel wall appeared deficient at the attachment of the thrombus (Fig. 9), which may be regarded as evidence of the preceding vascular lesion. Subsequent morphologic developments producing the characteristic lesions described and illustrated by all previous observers result from the tendency of the thrombi to propagate and the reactive proliferation of the endothelium to encompass and then to organize them (Figs. 10 and 11). As has already been mentioned, the majority of the thrombi in the tissues represent extensions of lesions originating outside of the plane of the section.

As the thrombi aged, variations in the structure occurred; the youngest form consisted purely of platelets (Fig. 12). Subsequently, small quantities of fibrin were deposited (Fig. 13), and proliferation of the endothelium took place at the margins of the attachment of the thrombus to the vessel wall (Fig. 9). The endothelial growth served first to re-establish the continuity of the intimal lining since it did not "invade" the thrombus until it had encompassed it and segregated it

from the circulation. Organization from the base of the thrombus did not occur, as would be expected if the endothelium beneath the thrombus had been intact (Fig. 14). With progressive organization, there was a mild reactive fibrosis extending into the adjacent perivascular stroma (Fig. 15).

Discussion

The variability of the endothelial reaction led many of the previous authors to conclude that there was no evidence of primary vascular disease.[1-4,6,10,11] A few others described rare foci of hyperplastic endothelium which they regarded as possibly the initial lesion.[3,5,9,12] To account for the formation of platelet thrombi such hypotheses were suggested as: (a) an excess of the antihemophilia globulin leading to increased agglutination and disintegration of platelets[9]; (b) an inadequacy of the normal blood antithrombin which would permit the presence of traces of active thrombin[9]; (c) the presence of a hypothetic toxin exhibiting thrombin-like activity, much like that of certain snake venoms[6]; or (d) the presence of a hypothetic toxin affecting endothelium.[4] With all of these theoretic mechanisms, however, thrombosis would be expected first at sites of slow circulation, namely, on the venous side, to produce syndromes of the type which plague clinicians treating a variety of debilitating and postoperative conditions. Moreover, the hypotheses are inadequate to explain the peculiar structure of the thrombi. Bernheim[6] failed to demonstrate an auto-agglutinin for platelets. On morphologic grounds, if such a substance were present, platelet thrombi, contrary to what is found, would be expected to be most numerous in such extensive capillary beds as those of the lungs and the liver.

Thrombophlebitis with platelet aggregates involving small veins and venules occurs in the eschar and rash of scrub typhus; infrequently, occluding platelet lesions of glomerular and pulmonary septal capillaries may be found.[13] The accompanying striking inflammatory reaction and the totally different basic pathologic features make the differential diagnosis a simple matter. Moreover, there is little likelihood that the arteritis with marked endothelial cell damage, prominent in epidemic typhus and in Rocky Mountain spotted fever, would be mistaken for the bland type of vascular lesion encountered in disseminated platelet thrombosis. It is worthy of note, however, that in these three rickettsial diseases, Allen and Spitz[13] found platelet thrombi in the one (scrub typhus) which exhibited the slightest degree of endothelial damage. Presumably, the more intense process causes platelets

adhering to the injured lining to disintegrate faster than they can accumulate.

Taking as a clue the correlation of the structure of thrombi in rickettsial diseases with differing degrees of vascular injury, a reasonable hypothesis may be constructed to explain the occurrence of platelet thrombi without invoking an exception to the widely accepted concept of blood coagulation.[14] Following an acute injury—for example, physical trauma—to vascular endothelium, platelets accumulate over the damaged area. Their disintegration, an effect of the injury (assuming the normality of the other blood-coagulating factors), initiates the chemical reactions which culminate in the focal formation of a fibrin net enmeshing and entrapping large numbers of blood cells. The number of blood cells that may be encountered depends upon the caliber of the vessel in which thrombosis is occurring. In vessels of capillary size, the platelet and fibrin components usurp a greater proportion of the lumen than in larger channels. The size of the clot within the confines of the vessel is limited by the presence of antithrombic substances in the plasma, and at the periphery of a stationary clot an equilibrium exists between them and the thrombin released at the site of injury. It is readily apparent how slowing or diminution of the circulation, by reducing the availability of antithrombin, permits the thrombus to propagate. With minor injuries of the order reported by Chambers and Zweifach[15] in micromanipulative studies of the capillary wall, adherence of platelets to the inner surface of the endothelium is temporary and reversible. Disintegration of thrombocytes and clotting, therefore, need not be, *pari passu,* a consequence of platelet aggregation. Inflammatory injuries of vessel walls must certainly introduce with the infiltrate a number of factors, fibrinolytic as well as antithrombic, to account for the relatively rare and only sporadic occurrence of intravascular clotting in inflammatory conditions. Holman[16] was impressed with the infrequency of thrombosis with the severe necrotizing arteritis he had produced experimentally. When such clot-inhibiting factors are absent or minimal, and the degree of intimal injury is such that thrombocytes accumulate much more rapidly than they disintegrate, the stage is set for platelet thrombosis.

The nature of the vascular injury is also a matter of speculation. A formed structure is normally absent from the subendothelial position occupied by the hyaline prethrombotic lesion. The positive Schiff staining reaction merely indicates that free aldehyde groups are present in the material.[17] However, the rapid swelling exhibited in progressing to the formation of a platelet thrombus suggests a relation to

the substance responsible for capillary permeability, which, according to Chambers and Zweifach,[15] is the cement substance intervening between adjacent endothelial cells. An alteration of this material, normally without coagulating activity, could readily provide the minor injury causing platelets to accumulate faster than they are destroyed. Abnormal stability of the thrombocytes, which would facilitate their accumulation, seems to be ruled out by the normal clotting times reported in this condition. Under normal circumstances, platelets are short lived structures,[18] but their physiologic disintegration produces no harmful effect because of the antithrombic substances in the plasma.[19] However, in a situation where large, occluding aggregates of platelets form, the access of circulating antithrombin is so restricted that fibrin may be precipitated even by the normal rate of platelet decomposition. The amount of fibrin cannot be large since the accumulations of platelets limit the availability of plasma; but at any rate it is unnecessary to postulate an exception to the clotting mechanism. The striking tendency of the lesions to propagate along the length of a vessel is attributable again to the reduced access of circulating antithrombin to the site of the occluding lesion.

In attempting to arrive at a better understanding, this syndrome has been compared with other established processes. Hog cholera, a virus disease of domestic swine characterized by widely scattered purpuric hemorrhages with pulmonary and enteric symptoms,[20] presents as its basic pathologic change, widely distributed thrombotic vascular lesions which have been likened to those in the disease under discussion. In hog cholera, however, there is a primary lesion of the endothelium with retrogressive changes extending to involve the entire vessel wall, and also an associated, inflammatory infiltration,[21] a feature strikingly absent in the early lesion of the human disease.

The relation to disseminated lupus erythematosus which has been suggested[10] appears to have little basis either clinically or pathologically.[3,7,9,22] Microscopically, the segmental hyaline thickening of the basement membrane of the glomerular capillaries, the "wire loop" lesion, has no counterpart in the much more widespread lesions of disseminated platelet thrombosis. Although occasionally a mass of material originating from the basement membrane may almost occlude a glomerular capillary in lupus, it may be readily distinguished from a platelet thrombus, nor does it lead to the formation of one. Neither the skin lesions, the nonbacterial endocarditis, nor the widely scattered foci of collagenous degeneration which characterize lupus[23] are to be found in disseminated platelet thrombosis. Occasionally, as in case 4

of this series, reactive fibrosis about thrombosed follicular arterioles in the spleen may produce a picture suggestive of the concentric, collagenous rings frequently present in lupus. Two cardinal features of the clinical picture, purpura and thrombocytopenia, characteristic of the one disorder would certainly be unusual in the other.

Baehr, Klemperer, and Schifrin,[1] in attempting to relate the syndrome to hypersensitivity, pointed to the occurrence of platelet thrombi in the Shwartzman reaction[24,25] and in the visceral capillaries of horses immunized with repeated injections of living bacteria.[26] However, such lesions involved venules and venous capillaries in contrast to the arteriolar localization in the disseminated human disease, and showed a striking inflammatory reaction in contrast to the bland process in platelet thrombosis. Experimental peptone shock and anaphylactic shock are characterized by a thrombocytopenia which Quick[27] has attributed to agglutination and disintegration of platelets, a consequence of the release of histamine into the circulation. Copley[28] has shown that hyperheparinemia, also an effect of shock of these types, produces thrombopenia in a similar fashion. Such mechanisms, however, cannot be considered as playing a rôle in the syndrome of disseminated platelet thrombosis, since, in the latter, the pathologic features rule out the occurrence of spontaneous agglutination of thrombocytes and since the normal clotting times observed are incompatible with elevated blood levels of an anticoagulant.

These distinctions from specific types of hyperergy and from disseminated lupus should not be considered to have eliminated the possibility of some other form of hypersensitivity reaction. Clinically suggestive evidence is present among the reported cases. In two, sensitivity to sulfa drugs was noted[3,29]; a third patient had had "hives" for 1 year requiring the administration of antihistaminic drugs.[3] In the case described by Muirhead, Crass, and Hill,[5] there was an associated diffuse proliferative glomerulitis, a lesion commonly looked upon as a result of hypersensitivity.[29] In case 3 of this series, subacute glomerular nephritis was present. Acquired hemolytic anemia, which may be a manifestation of sensitization,[30-32] was specifically mentioned in 3 cases,[5,12,29] but in view of the frequency with which jaundice is noted in platelet thrombosis (12 of 21 cases) as compared with other types of severe purpura, it probably occurred more often than the figures indicate.

Humble[33] made the interesting observation that petechiae in hemorrhagic diseases of varied etiology, both with and without thrombocytopenia, always occurred at the arteriolar-capillary junction. This

localization, curiously enough, is identical with that observed in the lesions of disseminated platelet thrombosis. In urticaria, too, the essential defect must involve the same segment of the vascular tree, since the rapidity with which wheals develop indicates the occurrence of filtration under high (arteriolar) pressure. We can accept Humble's conclusion that there is particular vulnerability of the arteriolar end of the capillary tree, but the evidence permits no inferences regarding the etiology of the syndrome of platelet thrombosis.

It is known that deficiencies of either vitamin C or calcium cause increased permeability of capillaries through their effect upon the intercellular cement.[17,34] But there is little ground for considering that either of these factors plays a part in the syndrome under consideration.

Summary

Five cases of disseminated arteriolar and capillary platelet thrombosis have been reported, bringing the total in the literature to 21. A focal vascular lesion of capillaries and arterioles demonstrated in these cases has been considered responsible for the development of the intravascular thrombi. The earliest prethrombotic lesion is non-inflammatory. Because of its tendency to undergo rapid swelling and its superficial subendothelial location it has been presumed to involve the cement substance which maintains normal vascular permeability. Rupture of the endothelium provides a focus upon which platelets accumulate at a rate faster than they are destroyed. The small caliber of the vessels involved limits the access of antithrombic substances so that propagation of the thrombus often occurs. Reactive endothelial proliferation starts promptly at the attachment of the thrombi and tends to encompass them. The resulting lesions are the ones most frequently seen in sections of autopsy material. Older lesions undergo organization and fibrosis and from the variability of those seen, it is inferred that they occurred intermittently in "showers." An alteration of the clotting mechanism was not observed in these cases and need not be postulated to explain the lesions.

The syndrome differs both clinically and pathologically from any of the well established disease entities. However, non-inflammatory involvement of arterioles and capillaries and increased permeability of these structures are features shared in common with urticaria and with hemorrhagic diseases of varied etiology.

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[*Illustrations follow*]

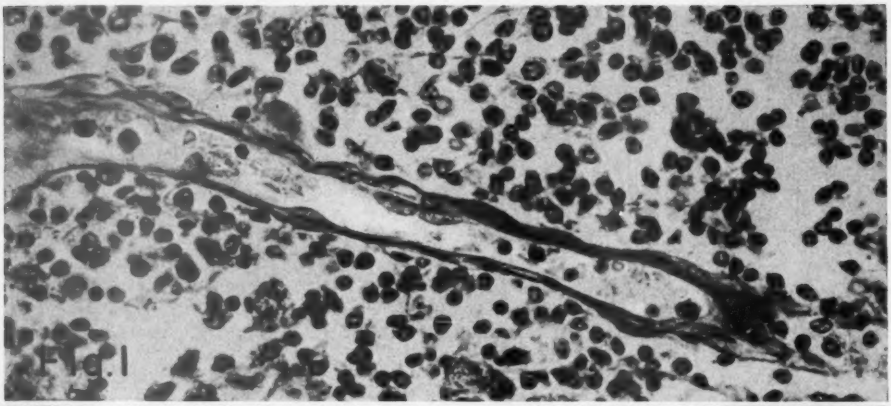
DESCRIPTION OF PLATES

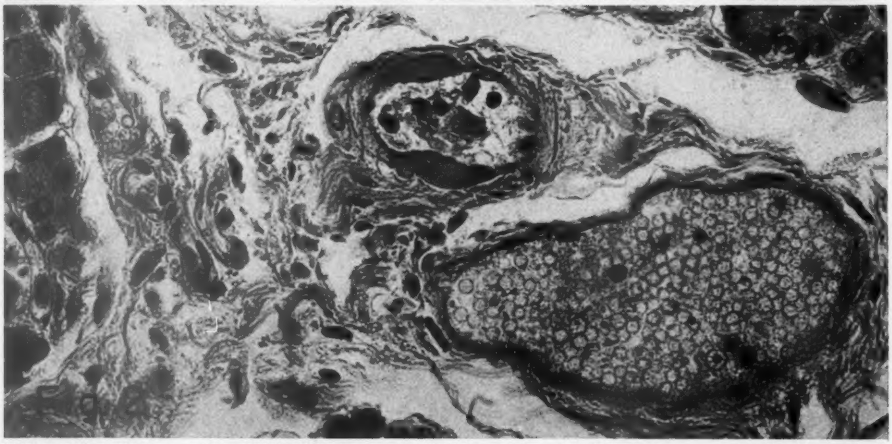
Plate 23

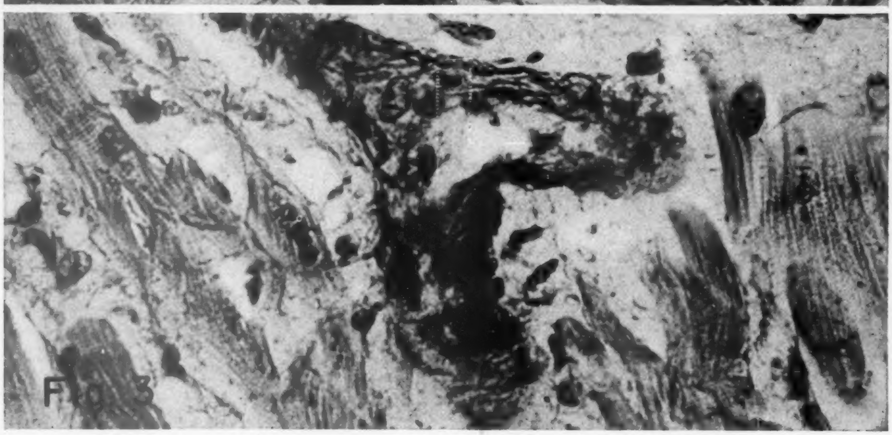
Fig. 1. Capillary vessel with a hilar lymph node exhibiting focal subendothelial deposition of homogeneous, pink-staining material, the prethrombotic lesion. Schiff periodic reaction. × 475. Armed Forces Institute of Pathology accession no. 193721, negative no. 104020.

Fig. 2. Arteriole in the substance of the pancreas. Focal subendothelial accumulations of hyalin, characterizing the prethrombotic lesion, may be observed. Schiff reaction. × 395. A.I.P. acc. 113583.

Fig. 3. Arteriole within the myocardium. Because of the tangential section, the focal hyaline lesion is not delineated as sharply as in the preceding figures. Schiff reaction. × 515. A.I.P. acc. 193721, neg. 104024.







Gore   Arteriolar and Capillary Platelet Thrombosis

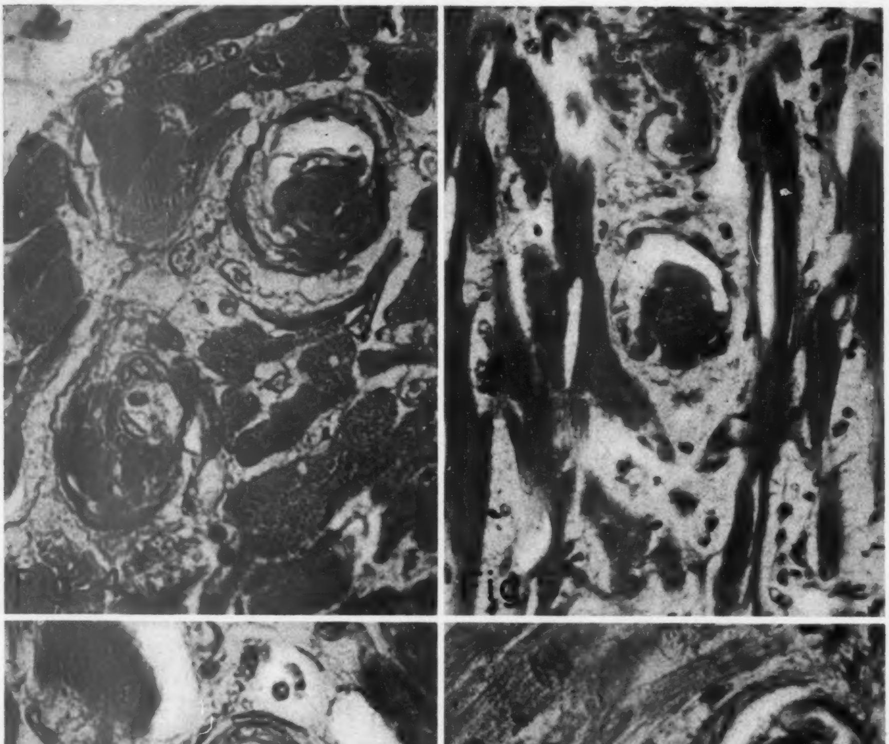
Plate 24

Fig. 4. Prethrombotic hyaline lesion bulging into the lumen of a myocardial capillary (?). There is an unaltered endothelial cell upon the summit of the projecting mass and the reticulin fibers, which are normal components of the vessel wall, show disruption and splitting. Schiff reaction. × 640. A.I.P. acc. 193721, neg. 104022.

Fig. 5. Prethrombotic lesion of a myocardial capillary protruding deeply into the lumen. Its position, in which it fills a defect of the vessel wall, creates the illusion that it is being extruded. The endothelium overlying the lesion has become attenuated, but at the margins there appears to be mild proliferation. Hematoxylin and eosin stain. × 400. A.I.P. acc. 121863, neg. 86142.

Fig. 6. Prethrombotic hyaline lesion of a myocardial capillary. The intact covering endothelium is not apparent in this optical plane. There is edema of the vessel wall adjacent to the nodule. Schiff reaction. × 640. A.I.P. acc. 193721, neg. 104021.

Fig. 7. Myocardium showing a capillary transected twice. In the larger segment the endothelium has been ruptured; the exposed surface of the hyaline lesion is covered with granular material (platelets). Of note is the absence of significant endothelial proliferation. In the smaller transected portion, the defect of the vessel wall at the site of the hyaline lesion creates the illusion of extrusion. The intact, but attenuated overlying endothelium is not visible in this optical plane. Schiff reaction. × 515. A.I.P. acc. 193721, neg. 104023.



Gore Arteriolar and Capillary Platelet Thrombosis

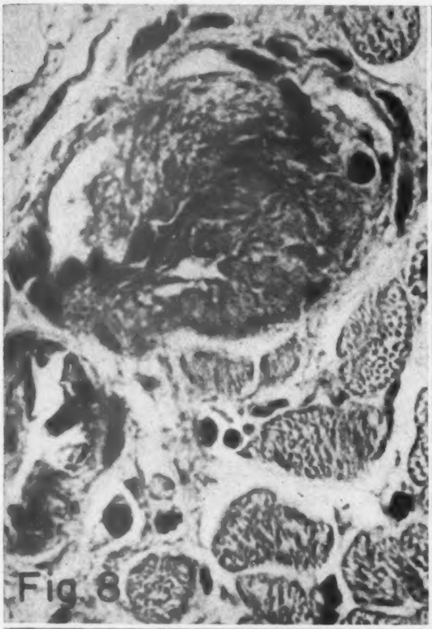
PLATE 25

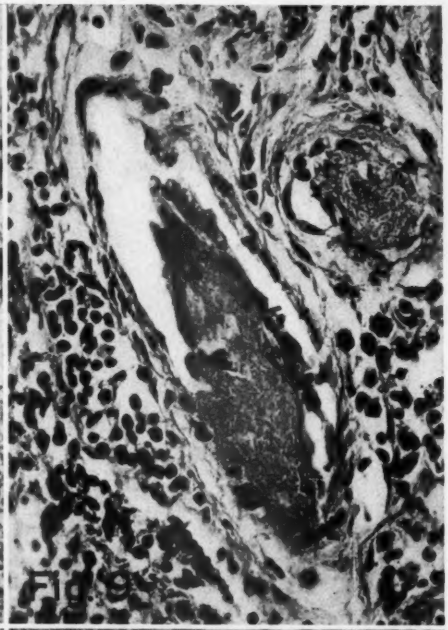
FIG. 8. Bimorphic thrombus. The homogeneous hyaline material occupying a defect in the wall of the vessel contrasts with the granular material (platelets) which have accumulated from the blood. There is proliferation of the endothelial cells at the margins of the thrombus, although the cells are absent at the interface between the hyaline and granular components. From the position of the adjacent arteriole it seems likely that the lesion lies in a segment of the same vessel. Heart muscle. Hematoxylin and eosin stain. × 640. A.I.P. acc. 100486, neg. 103974.

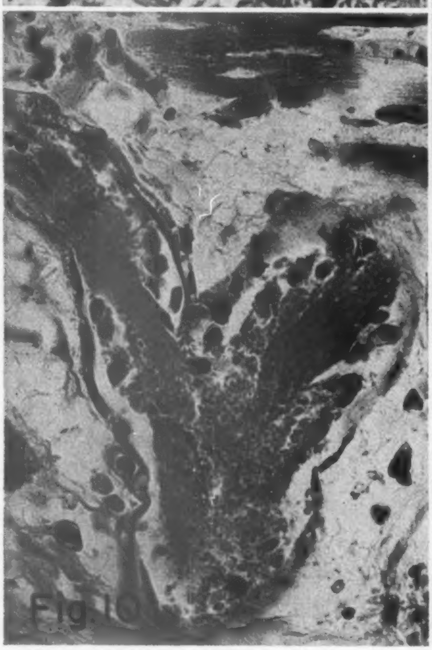
FIG. 9. Platelet thrombus showing early organization. A granular mass attached to a deficient segment of the vessel wall is enveloped and separated from the circulation by hyperplastic, actively proliferating endothelial cells, which contrast sharply with the normally thin and tenuous cells of the remainder of the lining. Lymph node. Hematoxylin and eosin stain. × 355. A.I.P. acc. 193721, neg. 104036.

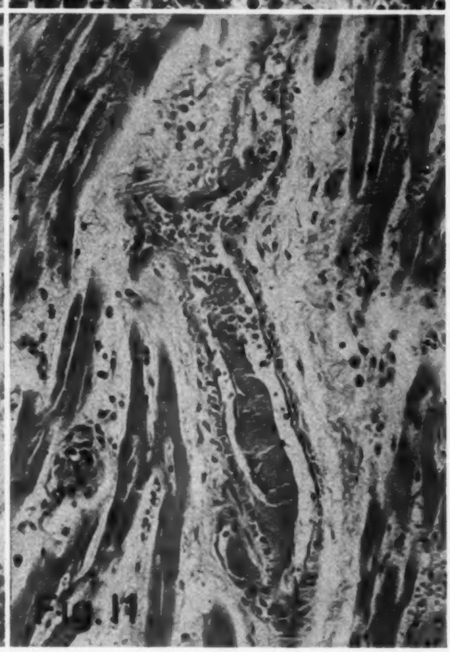
FIG. 10. Platelet thrombus extending the length of a vessel into its branches. The endothelial lining is unaltered; the scattered hyperplastic cells at the periphery of the thrombus must have some other source. Myocardium. Schiff reaction. × 395. A.I.P. acc. 113583, neg. 104011.

FIG. 11. Propagated platelet thrombus occupying an arteriole. The investing sleeve of proliferating endothelium is growing from the attachment of the thrombus. The vessel lining is otherwise unaltered. Myocardium. Hematoxylin and eosin stain. × 125. A.I.P. acc. 100486, neg. 103975.









Gore   Arteriolar and Capillary Platelet Thrombosis

PLATE 26

FIG. 12. Early platelet thrombus. The vessel is distended by an accumulation of thrombocytes which have entrapped two leukocytes. The vessel wall is defective at the attachment of the thrombus and endothelial proliferation is absent. Heart muscle. Schiff reaction. × 515. A.I.P. acc. 105882, neg. 104002.

FIG. 13. Platelet thrombus containing deposits of fibrin, probably at the distal extremity of a propagated lesion. The individuality of the platelets distinguishes this portion from the more compact proximal mass. The quantity of fibrin is limited by the availability of plasma. Myocardium. Schiff reaction. × 600. A.I.P. acc. 193721, neg. 104026.

FIG. 14. Platelet thrombus enveloped by endothelial cells, growing from the margin of its attachment to the vessel wall. The latter is deficient at that point and endothelial growth into the base of the lesion does not occur. Pancreas. Hematoxylin and eosin stain. × 555. A.I.P. acc. 110486, neg. 103985.

FIG. 15. Organizing platelet thrombus. There is a mild reactive fibrosis about the vessel. Lymph node. Hematoxylin and eosin stain. × 355. A.I.P. acc. 193721, neg. 104038.

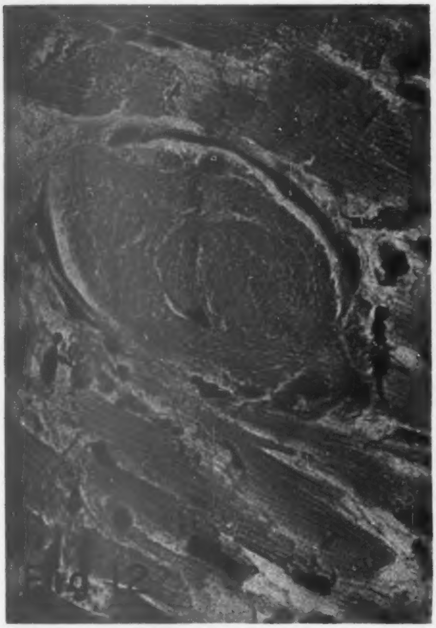


Fig. 12

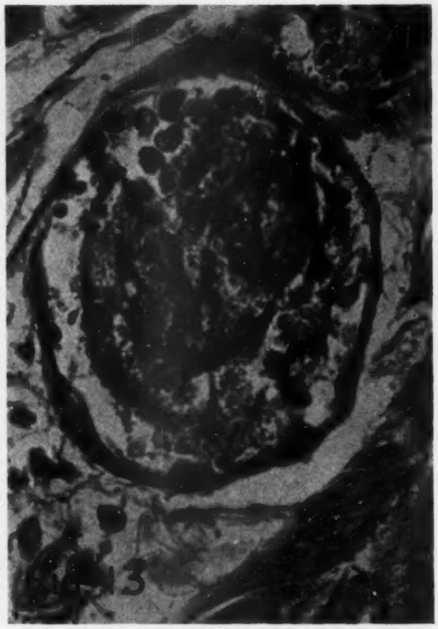


Fig. 13

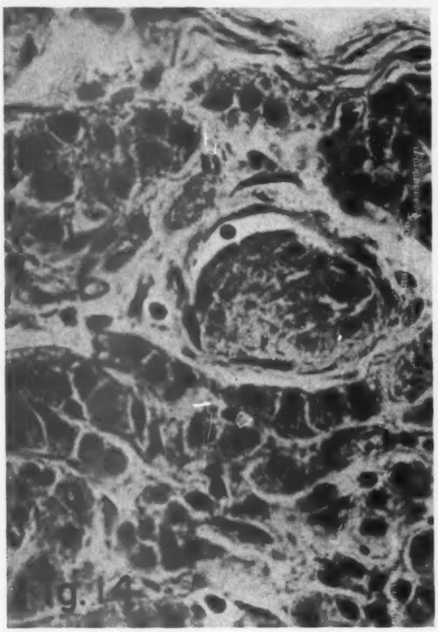


Fig. 14

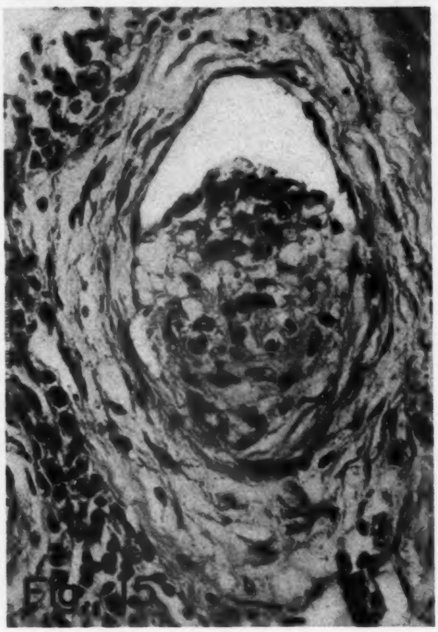


Fig. 15

Gore Arteriolar and Capillary Platelet Thrombosis